

Birla Central Library

PILANI (Jaipur State)

Class No :-

664.7

Book No :-

B15C

Accession No -

18032

THE CONSTITUENTS OF
WHEAT
AND WHEAT PRODUCTS

By

C. H. BAILEY

Professor of Agricultural Biochemistry

and

Director of the Agricultural

Experiment Station

The University of Minnesota



American Chemical Society
Monograph Series

REINHOLD PUBLISHING CORPORATION

330 WEST FORTY-SECOND STREET, NEW YORK, U. S. A.

1944

COPYRIGHT 1944 BY
REINHOLD PUBLISHING CORPORATION

All rights reserved

Printed in the United States of America by
THE HADDON CRAFTSMEN, INC., SCRANTON, PA.

GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the society, Washington, D. C.; the late John E. Teeple, then treasurer of the society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A. C. S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed editors (the present list of whom appears at the close of this introduction) to have charge of securing authors, and of considering critically the manuscripts submitted. The editors endeavor to select topics of current interest, and authors recognized as authorities in their respective fields.

The development of knowledge in all branches of science, especially in chemistry, has been so rapid during the last fifty years, and the fields covered by this development so varied that it is difficult for any individual to keep in touch with progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and by such compendia as Beilstein's *Handbuch der Organischen Chemie*, Richter's *Lexikon*, Ostwald's *Lehrbuch der Allgemeinen Chemie*, Abegg's and Gmelin-Kraut's *Handbuch der Anorganischen Chemie*, Moissan's *Traité de Chimie Minérale Générale*, Friend's and Mellor's Textbooks of Inorganic Chemistry and

Heilbron's Dictionary of Organic Compounds, it often takes a great deal of time to coördinate the knowledge on a given topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value. It was with a clear recognition of the usefulness of such work that the American Chemical Society undertook to sponsor the publication of the two series of monographs.

Two distinct purposes are served by these monographs: the first, whose fulfillment probably renders to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a form intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs enable such men to form closer contact with work in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made, and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, extended references to the literature enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection is made of those papers which are most important.

AMERICAN CHEMICAL SOCIETY

BOARD OF EDITORS

F. W. WILLARD, *Editor of Monographs.*

Scientific Series:—

S. C. LIND,
W. MANSFIELD CLARK,
LINUS C. PAULING,
L. F. FIESER.

Technologic Series:—

WALTER A. SCHMIDT,
E. R. WEIDLEIN,
W. G. WHITMAN,
C. H. MATHEWSON,
THOMAS H. CHILTON,
BRUCE K. BROWN,
W. T. READ,
CHARLES ALLEN THOMAS.

Introduction

This book started out to be a compilation of the significant facts and data relating to the substances present in wheat, and hence in its several structures, and its milled products. It was not intended to make it a handbook of analytical methods. Nor was there any intent on the part of the author to include what might be termed the dynamic biochemistry of wheat culture, wheat processing, or bread-making. Thus one would avoid including a consideration of the chemistry of the growth and development of the wheat plant, and the occasion for the variations observed in the percentage and nature of its constituents. It would also avoid including a discussion of the processing of wheat and of flour, which in turn, would involve the omission of references to enzymes and the mechanism of their action. In other words, this book should cover what might be termed the descriptive biochemistry of wheat and wheat products, leaving the dynamic biochemistry for treatment in another volume.

However, it was not easy to adhere strictly to such a program of writing. Thus the description of certain substances, notably the proteins, had to be presented in terms of the methods used in their fractionation, recovery, and purification. No sooner does one launch into detailed considerations involving the amounts and properties of the protein materials, than it becomes evident that these substances are not as definite and distinctive as, shall we say, the nature or amount of a simple inorganic salt in a mineral mixture. Accordingly, before the first chapter of the book had advanced more than a few paragraphs, methodology began to insinuate itself and could not be wholly thrust aside.

Likewise in certain other sections, dynamic biochemical considerations could be avoided only at the cost of omitting characterizations of some substances best recorded in terms of their behavior when hydrolyzed by enzymes. In the instance of selenium the temptation to outline the relationship between the amount and nature of selenium compounds in the substratum in which the wheat plant was grown, and the concentration in the tissues of the plant, particularly in the grain, was too great, especially since this is a subject which has made its appearance in the literature rather recently. So here again there was a little lapse from the ideal division of subject matter that was first contemplated.

Another sort of difficulty was also encountered, namely, the choice of the section or chapter in which certain facts or observations would be recorded. The outstanding example of this kind, so far as this book is con-

cerned, is the location of the discussion of the phosphorus-containing compounds. Rather than scatter this material through several chapters, the author chose to consolidate it in the phosphorus section of the chapter on minerals. This may seem strange, since phospholipids, phytin and the like are not minerals, though they all do contain at least one in common, namely phosphorus. But much of the data accumulated in consequence of their study is presented in terms of their phosphorus content, or the distribution of the total phosphorus between them. In other words, the element phosphorus has generally been kept in the foreground in the discussion of certain of these substances. On the other hand, nucleic acid and nucleo-proteins of wheat have been considered more particularly in terms of the organic groups present in them, and accordingly they are included in a special chapter among the proteins.

As in the instance of the author's earlier monograph, an effort has been made to exclude from consideration all materials or substances which are not present in natural wheat, even though they may be added to flour or other mill products in the process of manufacture. This policy involves the omission of references to flour bleaching and maturing agents, flour improvers, malt or malted wheat flour, flour-enriching ingredients, and the like. Since these materials are significant chiefly in terms of their role in the dynamic biochemistry of subsequent processing, or in metabolisms incidental to the use of wheat products as foods, their exclusion here is quite in keeping with the general plan of the book. Also many of them are present in wheat products essentially in the form and amounts in which they are introduced (certain gaseous bleaching and maturing agents excepted, of course) and hence need not be treated like those substances which owe their presence to the synthetic operations of the wheat plant itself.

The author anticipates that some who attempt to use this book may feel that it is too complete, if anything, in its coverage of certain items. Certainly one could pare down many sections if only the recent, more acceptable data and facts were retained. On the other hand, one's contacts with busy workers in these technological fields lead to the conviction that many of them do not have time or library facilities adequate to a perusal of the older literature, or that appearing in foreign journals. Accordingly, it seemed desirable to err on the side of completeness in certain sections rather than to trim the discussion and data down to facts which the author himself might chance to regard as the most acceptable.

In this same connection, there appears to be a lack of agreement among various investigators concerning what are the facts in the instance of various subjects covered in this monograph. It is only fair to present certain opposing viewpoints in such instances. Also, there may have been

actual differences in the composition and properties of the materials under discussion, which become evident only when all of the reasonably valid observations are compared. In fact, one may often find the key to the interpretation of some confusing observation by noting the actual variability among different samples of the same material which is evident from a consideration of all available data.

All of which may be taken to mean that the author did have a fairly definite plan in mind when this monograph was projected; that some departures from this plan have been deliberately or inadvertently made; that there are numerous sections or paragraphs included which could have been omitted or reduced in size, had historical developments, or controversial subjects been left out of the book altogether. It is hopeless to assume that the book will serve all the purposes of those who may consult it. Some can merely use it as a guide or index to the more detailed information contained in the published articles from which these condensed summaries have been gleaned. All must recognize that the sciences involved in the researches here recorded are still in the making, and this is merely a report of progress. A natural corollary of this statement is that the reader must endeavor to analyze certain apparently controversial issues for himself when contradictory evidence appears to be presented. And finally, the author wishes to record an appreciation of the stimulus to his own thinking and the extension of his own knowledge which has resulted from the encouragement given him to assemble such a manuscript. It has been an illuminating and intriguing experience which he hopes can be shared in some measure with his colleagues who use it.

C. H. BAILEY.

St. Paul, Minn.
December, 1943

CONTENTS

GENERAL INTRODUCTION	3
INTRODUCTION	5
1. EARLY RESEARCHES ON WHEAT PROTEINS	11
2. PROLAMINS OF WHEAT	30
3. GLUTELINS OF WHEAT	72
4. WATER- AND SALT-SOLUBLE PROTEINS	90
5. CRUDE PROTEIN AND CRUDE GLUTEIN	106
6. NUCLEIC ACID	128
7. NON-PROTEIN NITROGEN	131
8. STARCH	140
9. SUGARS	167
10. GUMS, PENTOSANS, HEMICELLULOSES, AND CELLULOSE	176
11. LIPIDS, PHOSPHOLIPIDS AND STEROLS	182
12. MINERALS	205
13. THE HALOGENS, SULFUR AND SELENIUM	240
14. ACIDITY	255
15. WHEAT AND FLOUR PIGMENTS	261
16. VITAMINS OF WHEAT AND WHEAT PRODUCTS	280
AUTHOR INDEX	319
SUBJECT INDEX	325

Chapter 1

Early Researches on Wheat Proteins

SECTION I. WHEAT PROTEIN RESEARCH 1728-1895

The choice of the time period through which the historical treatment of such a subject should run is purely arbitrary. The termination of the historical period might be dated at the time when the so-called "modern" work began. For convenience, the author elects to consider the researches of Osborne as belonging in the modern period, and, accordingly, will include in this historical section a summary of the literature published prior to the appearance of Osborne's work. The latter, because of its elaborate nature, and the effect which it had upon the later and recent studies in this field, will be made the subject of the second section.

It is generally conceded that the researches on wheat proteins date from the announcement by Beccari in 1728, and published in 1745 ¹ that he had effected the separation of gluten from flour by washing a dough with water. Beccari was a professor of medicine in the Anatomy and Chemistry Institute of Bologna, Italy. Evidently he had undertaken the task of investigating the nature of foods. As the discussion of the methods used by him is developed in the published report it becomes apparent that the facilities of the time were not very adequate or complete. Also the field of his interest and training in medicine obviously influenced his reasoning relative to the nature of gluten. Thus, after detailing how gluten was recovered from a refined flour by kneading with water to form a dough, and washing the starch and solubles from this dough with water, he proceeds to compare the gluten with animal products. Two general types of method were employed, a distillation, and a fermentation. The vegetable parts (starch), after a long-continued fermentation, produced an "acetic liquor with the savour of wine," whereas the glutenous material produced no characteristics of sourness but "putrefied strikingly like a dead body." On prolonged standing in contact with water the resulting fluid became turbid on treatment with mercuric sublimate and with a salt of lead; with alkali it did likewise. Moreover, a fine white powder settled out, which was attributed to the "volatile alkali" present. In these particulars the similarity to animal tissue, with which Beccari was

¹ "De Bononiensi Scientiarum et Artium Instituto Atque Academia," Volume 2, Part 1, pages 122-127 (1745).

familiar, led him to state that gluten "behaves as though of animal origin."

Likewise, when subjected to distillation at his hands, gluten yielded a succession of products similar to those previously obtained from animal tissue. First, water was recovered, then a vapor becoming yellow and also alkaline, next a yellow oily material from which snowy salts were deposited. Finally, when it was strongly heated, a dark, viscous oil with a bad odor was recovered. These, in general, were the products of distillation of animal products.

The "vegetable parts," doubtless chiefly starch, on distillation, yielded an acid vapor, which became increasingly acid on stronger heating, and later more reddish in hue; finally it was accompanied by a light oil which became progressively darker and more viscous, and floated on the aqueous portion of the distillate. Emphasis is laid upon the alkaline nature of the products of distillation and putrefaction of animal parts, as contrasted with the acidic materials appearing on fermentation or distillation of vegetable substances.

Obviously the thinking of the times did not permit of conceding that plants and animals might have some characteristics in common, in the matter of occurrence and significance of proteins in their tissues. The influence of this thinking persisted for a century and a half; long after Beccari the biochemists of the nineteenth century were still seeking terms derived from the nomenclature of the animal proteins to be used in describing "gluten fibrin," "gluten casein," and "mucedin," for example.

Apparently neither Beccari nor his contemporaries pursued these studies of the flour proteins further. It was not until 77 years after Beccari's notable discussion before the Academy of Bologna that Einhof² reported his observation that alcohol extracted a protein substance from flour. At that time he evidently assumed this substance to be identical with gluten.

This observation of Einhof's apparently inspired a renewed interest in gluten, and in flour proteins generally. It was followed by a series of investigations which, incidentally, led to a most confusing state of affairs, particularly respecting nomenclature.

One of the most extensive researches of this period was reported by Taddei³ (also spelled Taddey). He separated gluten into two protein preparations, *gliadine*, and *zimome*. They were obtained by kneading fresh gluten in successive portions of alcohol so long as that solvent continued to become milky when diluted with water. After decanting off the alcoholic solution from the settlings, the alcohol in the solution was per-

² *J. chim. phys.*, 5, 131 (1805).

³ *Ann. Phil.*, 15, 390 (1820).

mitted to evaporate slowly, leaving the concentrated *gliadine* in the form of a solution of the consistency of honey, mixed with a little yellow, resinous matter from which it was freed by digestion in sulfuric ether, in which the *gliadine* was not appreciably soluble. The portion of the gluten not dissolved by the alcohol was termed *zimome*.

Taddei's *gliadine*, when dried, was of a straw-yellow color, slightly transparent, brittle in thin flakes, and having a faint odor compared with that of baked apples. In the mouth it became adhesive, with a "sweetish, balsamy taste." In boiling alcohol it was fairly soluble, the solution losing its transparent appearance on cooling, and depositing an insoluble product. With distilled water, the dried *gliadine* was softened but did not dissolve; when treated with water at the boiling temperature it was converted into a froth, and the liquid became turbid.

An alcoholic solution of *gliadine* became milky when mixed with water, and was precipitated in white flocks by the alkaline carbonates. While scarcely affected by mineral and vegetable acids, it was dissolved in certain (diluted) acids and in aqueous caustic alkali solutions. It swelled when placed on hot coals and then contracted after the manner of animal substances. It burned with a lively flame and left behind a light, spongy char difficult to incinerate. While possessing some of the characteristics of the resins familiar to Taddei, *gliadine* differed from them in being insoluble in sulfuric ether. It was very sensibly affected by an infusion of nutgalls.

Zimome, as recovered and studied by Taddei, was the residue remaining after dissolving out the *gliadine* from gluten by repeated extraction of the latter with alcohol. Taddei remarked that this residue represented only about one-third the volume of the original gluten mass, due not only to the removal of the *gliadine*, but likewise to the loss of water. The *zimome* was further purified by either boiling in alcohol or by repeated treatment with cold alcohol until the extracts were free from *gliadine*.

The *zimome* thus recovered was in the form of small globules or in a shapeless mass, which was hard, tough, devoid of cohesion, and of an ash-white color. When treated with water it recovered part of its "viscosity" and became specifically heavier than water. It dissolved in dilute acetic acid (vinegar) and in the mineral acids at boiling temperature (concentration of these acids was not indicated). With caustic potash, it "combines and forms a kind of soap." When placed in lime water or in solutions of the alkaline carbonates, it became harder and assumed a different appearance without dissolving. When thrown upon red-hot coals it gave off an odor like that of burning hair or hoofs and burned with a flame.

Finally, Taddei ventured the opinion that *zimome* is to be found "in various parts of vegetables." He noted that it effects various kinds of fermentations, according to the nature of the substances with which it comes into contact. This doubtless accounts for the name assigned to it, which is derived from the Greek word for a ferment. This property of causing fermentation was not confined to *zimome*, however, according to Taddei's experience, because he specifically noted that his *gliadine* preparations were also capable of producing fermentation in saccharine substances.

This extended summary of Taddei's researches is included, not only because it is one of the classics of his time, but also because it demonstrates the advances in the methods of study which appeared during the early part of the nineteenth century in consequence of the availability of chemical reagents of known composition and higher purity.

About a decade later de Saussure⁴ extended these studies of the flour proteins and described a "plant-albumin" constituting about 72 per cent of the gluten, a "plant gelatin" for which he proposed the specific name *glutin*, and constituting about 20 per cent of the gluten, and 1 per cent of a mucin.

Berzelius⁵ apparently employed much the same descriptive terms as de Saussure. That portion of the gluten which was insoluble in alcohol he called "plant gelatin" because of its presumed similarity to albumin of animal origin. He also distinguished two proteins in the alcoholic extract of flour, to one of which he applied the term "mucin."

Even after these apparent proofs of the presence of more than one kind of protein in gluten, Boussingault⁶ did not distinguish between the entire gluten, and the alcohol-soluble protein of flour.

Liebig⁷ evidently did not accept Boussingault's view, however, and not only recognized the distinctiveness of the alcohol-insoluble portion of the gluten, but applied the term *plant fibrin* to it. This term may have been taken from the nomenclature of Dumas and Cahours, since one of them in "Lehrkursus von 1839" applied that term to the alcohol-insoluble residue of gluten. Also it appeared as the residue when gluten was treated with ammonia. The resulting turbid solution, on addition of acetic acid followed by boiling, gave a protein precipitate which Liebig termed *plant gelatin*. Concerning the properties of the latter, Liebig stated, "plant gelatin is a casein compound which differs from the plant fibrin through its solubility in boiling alcohol and the ease with which it can be dissolved by dilute ammonia at ordinary temperatures. If one heats the saturated

⁴ Schweigger's, *J. chem. u. physik.*, 69, 188 (1833).

⁵ "Lehrbuch der Chemie," *Aufl.*, 3, 6, 453 (1837). Cited by Osborne, p. 6. Original not seen.

⁶ *Ann. chim. phys.*, 65, 301 (1837).

⁷ *Ann. Chem. u. Pharm.*, 39, 129 (1841).

ammoniacal solution to boiling and adds acetic acid drop-wise, upon the neutralization which follows, a thick, white coagulum results, which does not differ from heated casein or other curd-like proteins."

Liebig also observed the presence of a protein in the water extract of flour, which he recovered and classified as an albumin.

Adequate quantitative methods having made their appearance during this period for the elementary analysis of organic substances, Liebig applied these methods to the three flour proteins which he or his colleagues had prepared, with the results recorded in Table 1. It will be observed

Table 1. Elementary Analyses of Plant Fibrin, Plant Gelatin, and Albumin from Flour, as Reported by Liebig.⁷

	Carbon	Nitrogen	Hydrogen	Oxygen + Sulfur + Phosphorus
Plant fibrin				
I. Dr. Jones	53.83	15.59	7.02	23.56
II. Dr. Scherer	54.60	15.81	7.30	22.28
III. Dr. Scherer	54.60	15.81	7.49	22.10
Plant gelatin (casein)				
Dr. Scherer	54.14	15.67	7.16	(O. S.) 23.04
Dr. Verrentropp	51.41	14.48
Plant albumin				
from wheat	55.01	15.92	7.23	(O. S. P.) 21.84
from rye	54.74	15.85	7.77	21.64
from plant gelatin	54.85	15.88	6.98	22.39
(Drs. Will and Verrentropp)				

that the percentage of nitrogen in these preparations was substantially lower than was later found in analogous preparations. This may have been due to an incorrect interpretation of the data accumulated in the analyses; in part to non-protein impurities in the preparations. Also the nitrogen content is more constant than in Osborne's proteins, for example.

Scherer⁸ then introduced a new technique into flour protein chemistry which involved dispersing the protein in dilute alkali, filtering, precipitating the protein by neutralizing with acetic acid, then extracting the precipitate with hot alcohol, and then with ether. The protein in the residue he regarded as a preparation of "plant-fibrin." Obviously the technique here applied approached in principle certain of the recent practices followed in fractionating and purifying cereal glutelins. Bonchardat⁹ also employed a dispersion method, involving very dilute acids, and decided that the gluten protein that was soluble in such reagents should be termed an "albuminose."

Dumas and Cahours¹⁰ probably launched the most elaborate protein

⁸ *Ann. Chem. u. Pharm.*, **40**, 1 (1841).

⁹ *Ann. Chem. u. Pharm.*, **42**, 124 (1842).

¹⁰ *J. prakt. Chem.*, **28**, 398 (1843).

fractionation procedure that had been applied to flour up to their time. They recognized four protein substances in flour: (1) "plant-fibrin," which constituted the residue from extracting gluten with alcohol; (2) a "casein-like" protein which precipitated upon cooling the alcoholic extract of gluten; (3) "gluten," which was obtained by concentrating the alcoholic extract, and (4) an "albumin" present in the aqueous washings of the gluten. The "plant-fibrin" had about the same ultimate composition as blood-fibrin and was assumed to be identical with the latter. Likewise the "albumin" of wheat appeared to be identical with egg albumin. Their thermal fractionating of the alcoholic extract is also noteworthy in view of the later application of related techniques to which reference is made under the section entitled *Prolamins*.

von Bibra's monograph,¹¹ which appeared in 1860, was really a monumental treatise on the chemistry of flour and bread, and detailed his extensive researches on the fractionation of the flour proteins and their composition. He recognized three proteins in gluten: (1) plant fibrin as named by Liebig, which constituted about 70 per cent, (2) plant gelatin ("Pflanzenleim"), 16 per cent, and (3) plant casein, 7 per cent. The composition of typical preparations was reported by him as follows:

	Plant fibrin	Plant gelatin	Plant casein
Carbon	53.67	53.56	54.88
Hydrogen	6.95	7.12	7.05
Nitrogen	15.70	15.56	15.70
Oxygen	22.66	22.88	21.68
Sulfur	1.11	0.88	0.69

von Bibra also observed the presence of an albumin in the water used for washing out the gluten, and reported the percentage, ranging from 1.33 to 1.50 per cent. These values are somewhat lower than those quoted by him from Peligot's analyses, which ranged from 1.4 to 2.4 per cent.

Günsberg¹² apparently accepted Taddei's conclusion that wheat gluten was composed of only two proteins. He boiled wheat gluten with water; on cooling, five preparations were recovered, all of which had about the same elementary composition as the *gliadine* preparations previously reported. *Gliadine* behaved in like manner.

Commaille¹³ apparently evolved a new nomenclature for the wheat proteins as follows: *sitosine* (albumin of flour), soluble in water and coagulable by heat; *inesine* (fibrin of gluten), dispersed after drying by dilute (0.1 per cent) HCl solution; *sitesine* (casein of gluten), soluble directly in the same reagent; *glutine*, practically insoluble in dilute, but easily dispersed by strong acid solutions, forming an emulsion with alcohol

¹¹ "Die Getreidearten und das Brod," Nürnberg, 1860.

¹² *J. prakt. Chem.*, 85, 213 (1862).

¹³ *J. Pharm.*, 4, 108 (1866).

from which it is precipitated by the addition of much water; and *mucine*, which dissolves in water and in cold 80 per cent alcohol. A consolidated tabulation of these characteristics is as follows:

Gluten:	Water	Alcohol	Dil. HCl	HgCl in solu. with HCl
Glutine	Insol.	Sol.		
Mucine	Sol.	Sol.	Difficultly sol.	Precipitated
Sitesine	Insol.	Sol. in cold	Sol.	None
Inesine	Insol.	Insol.	Difficultly sol.	Precipitated
Sitosine	Sol.	Insol.	Difficultly sol.	Precipitated

The elementary analysis of "Pflanzenleim," or plant gelatin, on the ash-free basis was found by Ritthausen ¹⁴ to be:

Carbon	52.6	per cent
Hydrogen	7.0	" "
Nitrogen	18.06	" "
Sulfur	0.85	" "
Oxygen	21.49	" "

The substance dissolved in concentrated HCl to a clear solution of blue color; in HNO₃ (sp. gr. 1.2) it dissolved after a short heating, became slightly turbid on cooling, and deposited yellow flocks when mixed with water. Dilute H₃PO₄ solution effected little change in the cold, but produced a turbid solution on heating which deposited the protein apparently unchanged when cooled. Saturated tartaric acid dissolved the protein when heated, and the dispersion remained unchanged on cooling. In acetic acid it dissolved readily in all proportions to a clear, faintly yellow solution. Dilute solutions of sodium and potassium hydroxide dissolved it readily, without change when cold, but with decomposition when heated. Ammonia was less effective as a solvent and the dispersion was never clear. Significant quantities could be dissolved by calcium and barium hydroxide solutions.

"Gluten casein" was recovered by Ritthausen ¹⁵ as a copper hydroxide complex which contained from 14.14 to 14.70 per cent of nitrogen and 14.0-15.23 per cent of copper. On the basis of ash-free material, the average composition of three gluten casein preparations was:

¹⁴ *J. prakt. Chem.*, **88**, 141 (1868).

¹⁵ *J. prakt. Chem. (new series)*, **5**, 215 (1872).

Carbon	51.49	per cent
Hydrogen	6.78	" "
Nitrogen	17.06	" "
Oxygen + Sulfur	24.67	" "

Although Ritthausen ¹⁶ had published a short paper on the constituents of wheat gluten in 1862, his excellent monograph published in 1872 ¹⁷ covered his detailed researches on the proteins of the cereals, as well as several legumes, buckwheat and rape seed. At the time of his work only six amino acids had been identified or discovered, *viz.*, leucine, glycine, tyrosine, serine, aspartic and glutamic acids. Incidentally, the last two were discovered by Ritthausen himself in 1868. Nor were adequate quantitative methods at hand for determining the concentration of all these substances in protein hydrolyzates, although he did report on the aspartic and glutamic acids. Accordingly, the protein preparations recovered by Ritthausen and his contemporaries could not be characterized in as definite terms as was possible early in the following century.

Ritthausen accordingly was obliged to resort to distinctions based upon the solubility of the wheat proteins, and the elementary analyses of the resulting preparations. He recognized five proteins and observed the proportions in which they were present. His nomenclature had much in common with von Bibra's, who, in turn, had accepted much of the earlier terminology.

Ritthausen's Protein Classification

I. Gluten casein. Insoluble in water, slightly soluble in dilute alcohol, readily dispersed by dilute acids and alkalis. On hydrolysis with sulfuric acid, tyrosine, leucine, glutamic acid (5.3 per cent) and aspartic acid were identified in the hydrolyzate. The average elementary analyses of two series of preparations are recorded in Table 2. From 100 parts of wheat gluten (dry basis) he recovered 28.3 parts of *gluten casein*, and 42.0 parts of alcohol-soluble proteins. The remaining 30 per cent comprised fat, starch, bran, and unidentified substances or losses due to manipulation.

II. Gluten-fibrin. Several proteins were extracted from wheat gluten by alcohol (60-80 per cent), including "Pflanzenleim" (plant gelatin) or *gliadine*, *mucedin*, and *gluten-fibrin*. Gluten was treated with hot 50-60 per cent alcohol, in the ratio of 8 to 10 times as much alcohol solvent as dry substance dissolved. This solution was concentrated, cooled, and the gluten fibrin separated out. While he recovered only 2-3 per cent on the basis of the dry gluten (equivalent to 0.25-0.35 per cent on the basis

¹⁶ *J. prakt. Chem.*, 85, 193 (1862).

¹⁷ "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen," Bonn, 1872.

of the original flour) as gluten fibrin, Ritthausen was confident that the actual proportion was much larger. Also he concluded that the content is quite variable among wheats of diverse types, being greater proportionately in the soft than in the hard wheats. He considered that the proportion in gluten affects the properties of the latter, and may even be involved in the ease and completeness with which it can be recovered. Thus the basis for the later researches of Fleurent, and of Snyder, to which reference will be made elsewhere, was laid in Ritthausen's observations.

Ritthausen further observed that when gluten fibrin was boiled with water it was so altered as to render it insoluble in alcohol. It could be extracted readily from the original gluten preparations by alcohol solutions of concentrations ranging from 30 to 70 per cent, and was even soluble to a considerable degree in 80-90 per cent alcohol. When concentrated solutions of the alcohol-soluble gluten proteins were cooled, the gluten-fibrin appeared upon the surface as a soft, thick film. When the first film was removed, it was renewed, a fact which made its quantitative separation virtually impossible, although Ritthausen considered that it was this property which distinguished it from mucedin and plant-gelatin or gliadin.

III. Plant gelatin (gliadin). Slightly soluble in cold water, more soluble in hot water. Freely soluble in 60-70 per cent alcohol, the solubility diminishing appreciably in departing from this range of solvent concentration. When boiled with water it lost the property of dissolving in alcohol. For reasons outlined under II above, its fractionation could not be effected quantitatively and, accordingly, Ritthausen did not attempt to estimate its concentration in wheat gluten. He did record a difference in the nitrogen content of the two alcohol-soluble proteins, plant gelatin, and gluten fibrin, however (see Table 2), while his mucedin preparation closely resembled the latter in that particular.

Ritthausen further emphasized that it is the gliadin which imparts coherence, tenacity, and ductility to wheat gluten; in his opinion it may be regarded as the binding material which holds together the other particles of the gluten. Kernels rich in gliadin may be expected to be hard and glassy.

IV. Mucedin. The third of the alcohol-soluble proteins of wheat gluten was the most soluble of the three in diluted alcohol, and in water, but otherwise did not differ greatly in its solubilities from plant gelatin or gliadin. It remained in the mother liquor of the alcohol-extract of gluten after removing the gluten-fibrin, and was the first precipitate formed when gliadin was recovered from its acetic-acid solutions on neutralizing the latter with alkali. Also it could be separated from gliadin by virtue of

its lower solubility in strong alcohol at ordinary temperatures. Ritt-hausen's purest mucedin preparation contained the lowest percentage of nitrogen of any of the flour proteins recovered by him, *viz.*, 16.63 per cent (see Table 2).

V. **Water-soluble protein (albumin).** This had been observed by other researchers, appearing in a coagulated form on heating the wash-water recovered on washing out crude gluten from a flour dough. Ritt-hausen prepared albumin in that manner, followed by the extraction of the fat, and drying the residue. It contained 4.64 per cent of ash and had the elementary composition recorded in Table 2.

Table 2. Elementary Analysis of the Five Protein Preparations
Reported by Ritthausen.¹⁷

	Gluten casein	Gliadin or plant gelatin	Gluten fibrin	Mucedin	Albumin
Carbon	52.94	52.76	54.31	54.11	53.12
Hydrogen	7.04	7.10	7.18	6.90	7.18
Nitrogen	17.14	18.01	16.89	16.63	17.60
Oxygen	21.92	21.37	20.61	21.48	1.55
Sulfur	0.96	0.85	1.01	0.88	20.55

Weyl¹⁸ identified a globulin in flour, in addition to a heat-coagulable "myosin." He credited Denis ("Memoire sur le sang," Paris, 1859, p. 171 ff.) with first identifying a *globuline* in wheat flour, soluble in 10 per cent NaCl solution, and designated as *glutine*.

Weyl and Bischoff¹⁹ considered the gluten to be derived from a globulin-substance or vegetable myosin which constituted the principal protein of flour. The process of conversion of myosin into gluten was assumed to involve an enzyme action analogous to that of the fibrinogen → fibrin conversion. An analogy between the two processes was believed by them to appear in the fact that pretreatment with NaCl, Na₂SO₄, and MgSO₄ inhibits or hinders the normal sequence. The fact that no gluten could be recovered from flour previously extracted with alcohol was interpreted to indicate that the myosin had been coagulated by the alcohol treatment.

Balland²⁰ took exception to the conclusion of Weyl and Bischoff, however. His experiments convinced him that no enzyme action was involved in gluten formation, and, moreover, that its constituents pre-exist as such in the wheat and do not appear from a "glutenogene." In general, this view was accepted by Johannsen,²¹ who further concluded that gluten as such constitutes the protoplasmic material of the starchy endosperm cells of the wheat kernel.

¹⁸ *Z. physiol. Chem.*, 1, 72 (1877).

¹⁹ *Ber.*, 13, 367 (1880).

²⁰ *Compt. rend.*, 97, 651 (1883).

²¹ *Ann. agron.*, 14, 420 (1888).

Martin²² found only one protein in the alcohol-soluble fraction of gluten, which was classified as an insoluble phytalbumose. It was insoluble in cold, but somewhat soluble in hot water. The protein in the alcohol-extracted gluten appeared to be uncoagulated, and could be dispersed in dilute acid and alkali solutions. Martin called this substance "gluten fibrin" in keeping with Liebig's terminology. For some peculiar reason, Martin concluded that direct extraction of flour with 76-80 per cent alcohol did not result in the removal of any protein, and, accordingly, he incorrectly reasoned that the insoluble phytalbumose does not exist as such in dry flour, but is forced from a soluble precursor by the action of water. At the same time the gluten fibrin was presumed to be formed from a precursor, the transition being described as a globulin \rightarrow albuminate conversion. This albuminate or gluten fibrin, plus the insoluble phytalbumose, then, constituted the gluten as recovered by washing a flour dough. Martin also observed a globulin and an albumose in the wash water collected from the gluten-washing operation.

The average composition of Chittenden and Smith's²³ several gluten-casein preparations did not agree very well with Ritthausen's¹⁷ findings. Thus the average nitrogen content was only 15.86 per cent, as contrasted with 17.14 per cent as reported by Ritthausen. In fact, none of Ritthausen's flour protein preparations contained less than 16.6 per cent of nitrogen, and it seems probable that the preparations of Chittenden and Smith were not freed completely from non-protein substances.

A protein named *artolin* was isolated from gluten by Morishimo;²⁴ it was soluble in dilute alcohol. He considered it to have been present as an impurity in Ritthausen's "Pflanzenleim" and in Osborne's "gliadin." The distinctions between these preparations, and quantitative methods for the recovery of this *artolin* are not made too clear in Morishimo's discussion, however. It seems probable either that protein denaturation was in progress, or that protein derivatives were formed which resulted in his securing what appeared to be a distinctive substance.

"Congluten" was found by Fleurent²⁵ to be a prominent constituent of durum wheat, comprising 15.66 per cent of the total protein, while gliadin represented 46.65 per cent, and glutenin 37.89 per cent.

O'Brien²⁶ identified a globulin, a proteose, and the gluten proteins in flour. His nomenclature differed from that of Osborne and associates, to which reference is made in the next section, to the extent that he labeled as a globulin of the myosin type the protein extracted from flour by dilute salt solutions and precipitated by later saturating the solution with

²² Brit. Med. J., Vol. II for 1888, p. 104.

²³ J. Physiol., 11, 410 (1890).

²⁴ Arch. exp. Path. Pharmacol., 41, 348 (1898).

²⁵ Compt. rend., 133, 844 (1901).

²⁶ Ann. Bot., 9, 543 (1895).

MgSO₄. This protein was considered to be an albumin ("leucosin") by Osborne *et al.* O'Brien considered that no ferment action was involved in gluten formation, and that, accordingly, a globulin did not constitute a precursor of gluten or any part of it. He labeled the alcohol-soluble protein of gluten *glian*, and the alcohol-insoluble fraction was called *zymom*, à la Taddei. The *glian* can be converted, according to O'Brien, into such derivatives as "myxon," "glutine," and "mucine," which, however, are not constituents of the native *glian*. This view was not accepted by Ritthausen,²⁷ who reiterated his earlier contention that there are three distinct flour proteins that are soluble in alcohol. No new evidence was offered by him in support of this contention, however.

Tabulation of Flour Protein Nomenclature as Accurately as is Possible in View of the Varying Methods of Preparation Employed (1820-1905).

	Gluten proteins	Non-gluten proteins
Beccari (1728)	Crude gluten	—
Einhof (1805)	Alcohol-sol. protein	—
Taddei (1820)	"gliadine" (alc-sol.); "zymome" (alc-insol.)	—
de Saussure (1833)	Plant albumin (72%); plant gelatin or gluten (20%); mucin (1%)	—
Berzelius (1837)	Alc-sol. proteins "mucin" and unnamed protein; alc-insol. "plant gelatin"	—
Liebig (1849)	Alc-sol. "plant gelatin"; alc-insol. "plant fibrin"	Water-sol. "albumin"
Scherer (1841)	Alkali- and alc-sol. protein; alkali-sol., alc-insol. "plant fibrin"	—
Bonchardat (1842)	Acid-dispersed proteins, collectively "albuminose"	
Dumas and Cahours (1843)	Alc-sol. "plant casein" pptd. on cooling; alc-sol. "glutine"; alc-insol. "plant fibrin"	Water-sol. "albumin"
von Bibra (1860)	"Plant gelatin" (16%) (alc-sol.); "plant casein" (7%) (pptd. on cooling); "plant fibrin" (70%) (alc-insol.)	* Water-sol. "albumin"
Commaille (1866)	"Sitosine," sol. in dil. HCl; "inesine," sol. in dil. HCl after drying gluten; "glutine," insol. in dil. acid, sol. in strong acid, pptd. by dil. of alcoholic ext.; "mucine," sol. in cold 80% alc.	"Sitosine" sol. in H ₂ O and coag. by heat.
Ritthausen (1872)	"Gluten casein," sl. sol. in dil. alc., sol. in dil. acids; "gliadin" or "plant gelatin," sol. in hot H ₂ O, alc-sol.; "mucedin," most sol. in alc., pptd. on neutr. an alkali sol. extr.; "gluten fibrin" pptd. on cooling a hot alc. extr.	Water-sol. "albumin"

* Where the term alcohol (or "alc.") is used, it implies ethyl alcohol ("weingeist" in the earlier German publications) diluted with water, generally to a concentration of 60% to 80% alcohol.

²⁷ *J. prakt. Chem.*, 59, 474 (1899). Cited by Osborne, p. 10. Original not seen.

Tabulation of Flour Protein Nomenclature—*Continued*

	Gluten proteins	Non-gluten proteins
		Globulin
Weyl (1877)		
Weyl and Bischoff (1880)	"Vegetable myosin," gluten precursor, and converted into latter by enzyme action.	—
Balland (1883)	Did not agree with Weyl and Bischoff	—
Johannsen (1888)	Supported Balland	—
Martin (1886)	Insoluble "phytalbumose," alc.-sol. and formed from a sol. precursor by action of water; "gluten fibrin," alc.-insol. and formed from a globulin by action of water.	—
O'Brien (1895)	Alc.-sol. "glian"; alc.-insol. "zymom" (see Taddei)	—
König and Rintelen (1904)	"Gliadin," sol. in 65% alcohol, insol. in 40% alcohol; "mucedin," sol. in 40% and 65% alcohol; "gluten fibrin" sol. in 65% and 90% alcohol; "gluten casein," insol. in 65% alc.	

König and Rintelen²⁸ recognized four distinct proteins in wheat gluten: (I) gluten casein, insoluble in 65 per cent alcohol, and three proteins (II, III, IV) soluble in 65 per cent alcohol, of which (II) gluten fibrin is soluble in 90 per cent alcohol, (III) mucedin is soluble in 40 per cent alcohol, and (IV) gliadin is soluble in 65 per cent alcohol and insoluble in 40 per cent alcohol.

As in all such treatments of the historical development of a field of science, arbitrary divisions of time must be employed. The present author has chosen to break this section with the work of König and Rintelen in 1904 because Osborne's classic monograph appeared shortly thereafter. Since Osborne's researches were so comprehensive, as contrasted with all that had been done previously, and since they exerted a profound influence upon the later studies, it has been deemed convenient to include them in the separate section of this chapter which follows. Also these data of Osborne's, and the later researches that are organized under the several headings of the individual proteins, may be regarded as part of the contemporary work, to the extent that we still employ much the same nomenclature and scheme of description of the flour proteins that was suggested by Osborne.

SECTION II. OSBORNE'S RESEARCHES ON WHEAT PROTEINS

The researches of Osborne and his colleagues prior to 1907 are conveniently summarized in his monograph, "The Proteins of the Wheat Kernel."²⁹ He describes in detail the chemical methods used in frac-

²⁸ *Z. Nahr. Genussm.*, 8, 401 (1904).

²⁹ "The Proteins of the Wheat Kernel," Carnegie Institution of Washington, 1907.

tionating the various amino acids which he identified, as well as the extraction and other procedures employed in recovering the several protein preparations which he studied. The latter may be summarized as follows:

Albumin ("leucosin"). Flour was extracted with aqueous 10 per cent NaCl solution, the extract saturated with $(\text{NH}_4)_2\text{SO}_4$, the precipitated protein dissolved in NaCl solution and then dialyzed until the globulin had been precipitated. After filtering, the albumin solution was coagulated by heating for an hour at $60^\circ\text{--}65^\circ\text{C}$. The coagulum was recovered, washed with water, alcohol, absolute alcohol, and ether, then dried to a horny mass. Preparations 1 and 2 were made in this manner.

Preparation 3 represented a protein coagulum recovered by heating the filtrate recovered after coagulating 2, to a higher temperature, *viz.*, 75°C .

Preparation 4 omitted the precipitation with $(\text{NH}_4)_2\text{SO}_4$, the filtered saline extract being dialyzed at once. After separating the globulin thus precipitated, the solution was heated to 90°C ., and the coagulum recovered and dried.

Preparation 5 was made from shorts, instead of flour. The saline extract from which the starch had been allowed to settle, was saturated with $(\text{NH}_4)_2\text{SO}_4$, the precipitate dissolved in 10 per cent NaCl solution, dialyzed to free it from globulins and the albumin coagulated at 65°C . The elemental analyses of these five preparations, on the ash-free basis, are recorded in Table 3.

Table 3. Ash-free Composition of Coagulated Wheat Albumin as Reported by Osborne.²⁰

Preparation:	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	Average (%)
Carbon	53.27	53.06	—	53.02	52.71	53.02
Hydrogen	6.83	6.82	—	6.87	6.85	6.84
Nitrogen	16.95	17.01	16.94	16.26	16.83	16.80
Sulfur	1.27	1.30	—	1.20	1.34	1.28
Oxygen	21.68	21.81	—	22.65	22.27	22.06

The agreement in composition was fairly good in all instances except Preparation 4, which contained appreciably less nitrogen than the other four. This was the preparation that had been heated to the highest temperature in coagulating the albumin.

Osborne then made seventeen preparations of albumin from wheat embryo meal, involving variations in the techniques of extraction and fractionation. Water was commonly employed as the extraction medium, followed in some instances by precipitation with $(\text{NH}_4)_2\text{SO}_4$, or saturated NaCl, and recovery by dialysis and heat coagulation. Many of these preparations contained considerable phosphorus, ranging as high as 6.73 per cent P_2O_5 . Accordingly, Osborne computed the elementary analyses on an ash-free basis, and concluded that the leucosin obtained from the

whole kernel has the same composition and general properties as from the embryo, although the former was free from phosphorus. Since a large quantity of albumin was required for studying the products of hydrolysis, wheat-germ meal was used because of the higher yields that could be conveniently obtained.

In the latter part of his monograph (p. 113) Osborne stated that leucosin constituted 0.3 to 0.4 per cent of the wheat kernel, whereas commercial germ-meal contained about 10 per cent.

The products of hydrolysis as determined by Osborne, are reported in Table 5 at the close of this chapter.

Globulin. Globulin was extracted with 10 per cent NaCl solution from straight grade flour; the extract was filtered, and proteins precipitated by saturating with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was then dissolved as far as possible in 10 per cent NaCl solution, filtered, and subjected to dialysis. The precipitate was recovered, redissolved in 10 per cent NaCl solution, and reprecipitated by saturating with $(\text{NH}_4)_2\text{SO}_4$. This was Preparation 23.

Another preparation was made in the same way, except that the original extract was dialyzed at once without the first precipitation with $(\text{NH}_4)_2\text{SO}_4$. This was Preparation 24.

Globulin was prepared from wheat shorts in much the same manner as Preparation 23 from flour. This was Preparation 25. Two additional fractions were obtained in this process, Preparation 26 from the filtrate and washings after dialysis by adding a few drops of NaCl and recovering the precipitate. The filtrate from this preparation was then heated to boiling, and the coagulum labeled Preparation 27. As will be seen by the data in Table 4 its nitrogen content approximated that of the other preparations, and all of these five preparations contained considerably more nitrogen than the albumins.

Table 4. Elementary Analysis of Five Wheat Globulin Preparations, Nos. 23 and 24 from flour, Nos. 25, 26 and 27 from Shorts, as Prepared and Analyzed by Osborne.²⁰

Preparation:	23 (%)	24 (%)	25 (%)	26 (%)	27 (%)	Average (%)
Carbon	51.07	51.01	51.00	—	—	51.03
Hydrogen	6.75	6.97	6.83	—	—	6.85
Nitrogen	18.27	18.48	18.26	18.64	18.29	18.39
Sulfur }	23.91	0.71	0.66	—	—	0.69
Oxygen }		22.83	23.25	—	—	23.04

As in the instance of leucosin, globulin was present in larger proportions in the wheat embryo than in flour. Osborne recovered ten preparations from germ meal, involving variations in the scheme of fractionation. The elementary composition of these preparations was quite similar to that of the flour globulin, the nitrogen content ranging between 18.41

and 18.85 per cent, averaging 18.58 per cent. Owing to the difficulties encountered in preparing large quantities of globulin, Osborne did not attempt to study the products of its hydrolysis. He reported (p. 115) that the wheat kernel contains about 0.6 per cent of globulin, the embryo or germ-meal about 5 per cent.

Proteose. In the extract freed from globulin by hydrolysis, Osborne recovered proteins representing one or more proteoses. These were precipitated by saturation with NaCl, or by adding 20 per cent of NaCl to the solution together with a little acetic acid. The quantity involved is very small and Osborne (p. 116) concedes that it was not definitely ascertained whether it was an actual constituent of wheat, or was formed from other proteins during their extraction and isolation. Two proteose preparations recovered by him were found to contain 17.08 and 16.89 per cent of nitrogen respectively. Proteoses were also recovered from the saline solution extracts of wheat-germ meal.

Gliadin (a prolamin). This protein was found to be slightly soluble in water, soluble in dilute alcohol, the solubility increasing until a certain concentration of alcohol is reached, and then becoming less soluble until it no longer dissolves in absolute alcohol. Osborne did not determine the concentration of alcohol which dissolved the largest proportion of gliadin, but decided that the maximum solubility is attained with about 70 per cent of ethanol by volume. As a matter of fact, Osborne's preparations were commonly made with ethanol-in-water of sp. gr. 0.90, which is somewhat less than 70 per cent ethanol by volume.

Gliadin proved to be soluble in other alcohols, including methyl, ethyl and benzyl alcohol, also in phenol, paracresol, and glacial acetic acid. Reference will be made later, in the section on prolamins, to the "solubility" of "gliadin" in various solvents, and at different concentrations.

Osborne also observed that gliadin could be dispersed by dilute acid or alkaline solutions, from which it could be recovered virtually unchanged by neutralizing these solutions.

The specific rotatory power of gliadin in 80 per cent alcohol was found to be $\alpha_D^{20} = -92.3^\circ$.

The elementary analysis, and products of hydrolysis of gliadin as determined by Osborne, are recorded at the end of this section in Table 5.

Glutenin (a glutelin), was previously described by Taddei under the name of "zimome"; by Liebig as "plant fibrin," which name was adopted by several later researchers; and by Ritthausen as "gluten casein." The name "glutenin" was proposed by S. W. Johnson, and was adopted by Osborne because the term "zimome," though entitled to adoption on the score of priority, is derived from the Greek word for a ferment, and implies that glutenin appears in consequence of a fermentation process.

The term glutenin serves to identify it as a prominent constituent of gluten.

Osborne's glutenin preparation appeared to be essentially insoluble in water and cold alcohol, although it was slightly soluble in hot alcohol and precipitated from the latter on cooling. When freshly prepared and in a hydrated condition it was readily dispersed in dilute acids and alkalies (*i.e.*, aqueous 0.1 per cent KOH solution). One of his preparations was made by first removing the proteins from flour that could be extracted (1st) with 10 per cent NaCl solution, followed by (2nd) 70 per cent (\pm) alcohol, and then extracting the residue for several days with 0.1 per cent KOH solution, filtering off the latter, and precipitating the glutenin by neutralizing with dilute HCl. When larger quantities were desired, he had recourse to crude gluten, which was recovered by washing a wheat flour dough with water to remove most of the starch and soluble material. The gliadin was then extracted with ethanol (sp. gr. 0.90), and the glutenin dispersed and precipitated as just described. The precipitation was commonly repeated to effect greater purification. Preparations (Nos. 93, 94, 95) resulting from successive reprecipitations did not differ significantly in elementary composition.

Osborne emphasized the difficulty of effecting filtration of the dispersed glutenin unless the lipoids had been removed previously by thorough extraction of the crude glutenin with alcohol and ether.

The elementary analysis and products of hydrolysis of characteristic glutenin preparations as determined by Osborne, are recorded in Table 5.

Hydrolysis of the Wheat Proteins (Osborne)

Osborne evidently employed a variety of techniques in an effort to recover a high yield of the several amino acids from the wheat protein preparations with which he worked. In several instances, notably with the considerable quantities of gliadin and glutenin that were available, he hydrolyzed nearly a kilogram of the protein with about 2 liters of a mixture of equal parts of concentrated HCl and water. This was heated for several hours on a water bath; then the temperature of the mixture was elevated to the boiling point in an oil bath and held there for 9-10 hours. It was then cooled with ice and saturated with HCl gas. After several days, glutaminic acid hydrochloride crystallized out and was recovered. Ammonia was recovered from the mother liquor by heating with an excess of barium hydroxide. Because of the high correlation of ammonia, and glutaminic (plus aspartic) acid content of a series of protein hydrolyzates, Osborne was apparently of the opinion that these acids occurred in the proteins in the form of acid amides which hydrolyzed, yielding the ammonia. Later researches indicated that ammonia may appear from

other sources in the hydrolyzate, and vary in consequence of the conditions, including duration, of hydrolysis.

It is not the purpose of this monograph to trace the details of the various methods used in the study of these hydrolyzates. Osborne used the best methods then known to him, which were based largely upon the practices of Emil Fischer and the techniques employed by Kossel and Kutscher, particularly in working over the bases. In certain instances, hydrolysis was effected with sulfuric acid solutions, presumably to parallel certain practices of Kutscher, and it was noted, for example, that the yield of glutaminic acid from such hydrolyzates was only 25.3 per cent, as compared with an average of 36.0 per cent received from the HCl hydrolyzates.

In the instance of the data recorded in Table 5 it appears that Osborne employed the uniform practice of calculating the yields as the free amino acid. Also that the percentages indicated are derived by dividing the weight of the free amino acid recovered by the weight of the protein

Table 5. Elementary Analysis and Products of Hydrolysis of the several Proteins as Reported by Osborne.²⁰

	Leucosin (an albumin)	Globulin	Gliadin (a prolamin)	Glutenin (a glutelin)
Carbon	53.02	51.03	52.72	52.34
Hydrogen	6.84	6.85	6.86	6.83
Nitrogen	16.80	18.39	17.66	17.49
Sulfur	1.28	0.69	1.03	1.08
Oxygen	22.06	23.04	21.73	22.26
Nitrogen factor N×	5.95	5.44	5.66	5.72
Glycocoll	0.94	—	0.00	0.89
Alanine	4.45	—	2.00	4.65
Amino-valerianic acid	0.18	—	0.21	0.24
Leucine	11.34	—	5.61	5.95
α-proline	3.18	—	7.06	4.23
Phenylalanine	3.83	—	2.35	1.97
Aspartic acid	3.35	—	0.58	0.91
Glutaminic acid	6.73	—	37.33	23.42
Tyrosine	3.34	—	1.20	4.25
Lysine	2.75	—	0.00	1.92
Histidine	2.83	—	0.61	1.76
Arginine	5.94	—	3.16	4.72
Ammonia	1.41	—	5.11	4.01
Tryptophane	+	—	+	+
Serine	—	—	0.13	0.74
Cystine	—	—	0.45	0.02

substance that was hydrolyzed and multiplying the quotient by 100. These data, accordingly, must not be confused with certain other tabulations where the percentage of total nitrogen of the protein charge that is present in each fraction is recorded.

Osborne's total recovery of amino acids was rather low, representing

50-60 per cent of the total nitrogen in the protein. Humin and tryptophane nitrogen are not reported, and these would have added appreciably to the total.

Despite the inadequacies and errors of these methods, it appears that Osborne's preparations must have involved materials of widely varying chemical constitution. Thus the leucosin preparation was low in total nitrogen content, yielded much less ammonia and glutaminic acids than gliadin and glutenin, but more lysine, histidine, and arginine (bases), as well as leucine, phenylalanine, and aspartic acid.

As between gliadin and glutenin, Osborne's hydrolyzates contained more ammonia and glutaminic acid when prepared from gliadin. Also glycocoll and lysine were not identified in the gliadin hydrolyzate, and the proportions of tyrosine and histidine were significantly lower. These data should be compared with the results obtained by other methods that are recorded in the sections which follow.

Osborne's researches did not include any extended attention to the development of quantitative methods for the determination of these proteins, or the amounts or proportions of them present in wheat or in different classes and grades of flour. He did recognize, and take advantage of the relatively large proportion of albumin and globulin in wheat germ. However, his practices in recovering protein preparations did serve as a basis for later studies on the part of other workers in the effort to develop quantitative fractionation techniques.

Chapter 2

Prolamins of Wheat

Following the work of Osborne, which was presumed to have established that gliadin of flour was a separate entity, food analysts settled down to the determination of gliadin as a more or less routine procedure. A contemporary of Osborne, Emil Fleurent,¹ working in Paris, employed a somewhat more elaborate method for the separation of gliadin, which is described in the next section. In the author's opinion, and particularly in the light of later studies, it is doubtful if Fleurent was dealing with the same substances in the final solution as would be encountered among the products of direct extraction with (neutral) alcohol. On the basis of these studies Fleurent concluded that the optimum ratio of gliadin to glutenin is 75:25; the outside limits would be 82:18, and 66:34.

Osborne apparently made little attempt to determine the quantity of gliadin in different wheats and wheat flours but quotes (his monograph, p. 111) the work of others. Thus Teller was reported to have found from 2.85 per cent in a white winter wheat to 8.15 per cent in a hard red spring wheat, this being 42.5 per cent and 34 per cent respectively of the total protein. Also Shepard was quoted as finding from 40 to 50 per cent of the total protein as gliadin in representative hard and soft wheats.

Snyder,² working at the Minnesota Agricultural Experiment Station, employed a direct extraction of flour with 70 per cent alcohol, and concluded that a well-balanced gluten is composed of approximately 65 per cent of gliadin and 35 per cent of glutenin. He later³ developed a polarimetric method of estimating the gliadin content of flour, and decided that the limits of gliadin in good baking flours were between 55 and 65 per cent of the total protein.

It was suggested by Guess⁴ that the gliadin-glutenin ratio might be accepted as a quality factor, while the percentage of gluten should be considered as a quantity factor in predicting the baking quality of bread flours. The first, multiplied by the second, might constitute a more acceptable basis of prediction than either taken alone, a view that was

¹ *Compt. rend.*, 123, 327: 755 (1896).

² *Minn. Agr. Exp. Sta. Bull.* 63, p. 519 (1899).

³ *J. Am. Chem. Soc.*, 26, 263 (1904).

⁴ *J. Am. Chem. Soc.*, 22, 263 (1900).

accepted essentially by Norton.⁵ Kosutany⁶ contended that the gliadin-glutenin ratio was of prime importance. Martin⁷ amended the gliadin figure by deducting the percentages of protein in a water extract from the percentage of alcohol-soluble protein. The resulting difference, or amended gliadin figure, was positively correlated with gas-retaining capacity of dough and superior bakers' scores.

Crude gluten was recovered from four samples of flour by Kosmin and Popzowa,⁸ and subjected to the separation of gliadin and glutenin. Two of these flours were described as "American Export Flours," (I) "Manitoba," and (II) "Buffalo," while the other two were "0-70 per cent extraction flours from hard wheat," Nos. III and IV respectively. The crude glutes were quite different in properties when recovered from the fresh flour Nos. I and II. It was elastic and coherent, rating No. 2 on Dr. Kosmin's scale, while the crude gluten from Nos. III and IV was originally an elastic gel which became weaker on standing, rating No. 3 on the same scale. Flours I and II were evidently of the high-protein type, yielding 14.9 per cent and 13.6 per cent of dry gluten respectively, whereas flours III and IV only yielded 9.5 per cent and 8.7 per cent. Gliadin in the glutes was determined by two methods: (A) direct extraction from the dried gluten by 70 per cent alcohol and determination of nitrogen in the alcoholic extract; (B) dispersion of the entire gluten in 0.2 per cent KOH dissolved in 79 per cent alcohol, followed by precipitation of the glutenin by neutralizing with HCl, using bromthymol blue as the indicator. The glutenin precipitate was recovered by centrifuging and washed again with 70 per cent alcohol. Gliadin was recovered from the neutral alcoholic solution by dilution with water. The precipitated gliadin was redissolved in 70 per cent alcohol, made to known volume, and the nitrogen content determined by Kjeldahling an aliquot. This last method gave slightly higher results, the difference being slight, however. The reverse was true, perforce, in terms of glutenin content.

In the instance of these two pairs of flour samples, the percentage of gliadin in the crude gluten was quite similar in the two strong flours I and II, being 54.8 and 52.5 per cent respectively, as determined by method A, and 51.3 and 50.5 per cent by method B. Using method A, weak flour III gluten contained 43.4 per cent, and IV contained 59.8 per cent gliadin. This represents a wider range than was encountered by other investigators, notably Blish,¹⁴ Zinn,¹⁵ Sharp and Gortner,¹⁶ and Grewe and Bailey,¹⁸ when analyses were confined to the original flour. While crude gluten may be a convenient source of gluten proteins at times, the author

⁵ *J. Am. Chem. Soc.*, **28**, 8 (1906).

⁶ *Proc. 7th Intern. Cong. Appl. Chem.*, **VIa**, 56 (1909).

⁷ *Brit. Med. J.*, Vol. II for 1886, p. 104.

⁸ *Mühlenlab.*, **4**, 153 (1934).

is in doubt concerning the acceptability of a quantitative method for gliadin and glutenin based upon extraction of them from crude gluten. There are too many opportunities for alteration of the ratio by differential solubility of the gluten proteins in the wash water, since gliadin is evidently dispersed by water and even by saline solutions. Also hydrolytic changes in these proteins may occur in preparing, holding and washing the dough which alter their "solubilities" when fractionization procedures are applied later. Finally, there is always an element of doubt as to what ensues when the gluten is dried: if heat is applied, denaturation ensues; if dried at room temperature, even *in vacuo*, as in Kosmin's and Popzowa's experiments, the process is so slow as to afford added opportunity for hydrolysis and consequent changes in the manner of dispersion, or subsequent solubility of the proteins.

The contention for an optimum ratio of gliadin to glutenin was supported by Pratolongo,⁹ however, even as late as 1938. Of course the issue becomes still more confused by the rising doubt as to the absolute identity of these two proteins, to which reference will be made in the closing paragraphs of this discussion of the prolamins.

It is not surprising, therefore, that the technological laboratories attached to the milling and baking industries were largely engaged in gliadin determinations during the period from 1900 to 1910. Gradually, however, disturbing scepticisms began to be expressed from numerous sources. König and Rintelen,¹⁰ Wood,¹¹ Shutt,¹² Ramstedt¹³ and others evidenced doubt as to the acceptability of the gliadin-glutenin ratio as a criterion of baking behavior. Blish¹⁴ suggested that the ratio might be less variable than had previously been assumed. Zinn¹⁵ computed the correlation between the percentage of gliadin and glutenin in a series of flours, and found it to be so nearly perfect as to suggest that one varied directly with the other. The data of Sharp and Gortner¹⁶ also disclosed a relatively constant ratio of glutenin to total protein in flours of widely varying composition and characteristics, and this would imply a similarly constant proportion of gliadin as well. This was also noted when the author recalculated the data of Blish and Sandstedt.¹⁷

Grewe and Bailey¹⁸ determined gliadin by first extracting flour with 5 per cent K_2SO_4 solution, and then extracting the residue with 70 per cent alcohol, using constant agitation during extraction. The preparation

⁹ *Chimica e industria*, (Italy), 20, 129 (1938).

¹⁰ *Z. Nahr. Genussm.*, 8, 721 (1904).

¹¹ *J. Agr. Sci.*, 2, 189, 267 (1907).

¹² *Proc. 7th Intern. Cong. Appl. Chem.*, VII, 108 (1910).

¹³ *Z. ges. Getreidew.*, 1, 286 (1909).

¹⁴ *J. Ind. Eng. Chem.*, 8, 138 (1916).

¹⁵ *J. Agr. Res.*, 23, 529 (1923).

¹⁶ *Minn. Agr. Exp. Sta. Tech. Bull.* 19 (1923).

¹⁷ *Cereal Chem.*, 2, 191 (1925).

¹⁸ *Cereal Chem.*, 4, 230 (1927).

was when whirled in a centrifuge to precipitate the insoluble residue. The clear supernatant liquid was decanted into a Kjeldahl flask, and the residue again treated twice with alcohol as before. The three extracts were combined in the same flask, the alcohol and water distilled off, and the nitrogen determined. This value multiplied by 5.7 was recorded as gliadin (I). Glutenin (II) was determined by the method of Sharp and Gortner to be described in the next section. In the instance of a series of 16 vulgare wheat flours the ratio $\frac{II}{I+II}$ (which is the ratio of glutenin to the sum of gliadin plus glutenin) ranged between 0.44 and 0.49 in every instance except one, when it was 0.41. This is so nearly a constant ratio, in view of the errors of such determinations, as to indicate again that the gliadin-glutenin ratio is not likely to constitute an index of other significant properties of flour.

A scheme of fractionating the flour proteins was employed by Javillier and Djelatides.¹⁹ Briefly summarized, the procedure was as follows:

- N₁. Dosage of nitrogen soluble in 10 per cent aqueous NaCl solution.
- N₂. Dosage of nitrogen in the residue from I which is soluble in neutral 70 per cent ethanol.
- N₃. Dosage of nitrogen in the residue from II which is dispersed by 0.3 per cent KOH solution.
- N₄. Residual nitrogen.

Various mathematical treatments of the resulting data were employed in the instance of data secured by the analysis of five French flours, containing from 1.55 to 2.12 per cent nitrogen, the latter being a durum wheat flour. These data are recorded in Table 6, and it appears that the ratio N₂/N₃, that is, the proportion of protein soluble in neutral 70 per cent ethanol to that soluble in 0.3 per cent KOH, was deemed to be of significance in predicting baking quality. This ratio may not be vastly different from the gliadin-glutenin ratio as determined by other investigators.

Incidentally, the ratios of N₂/N₄, as computed by the present author for the five flours, were found to be 0.348, 0.450, 0.453, 0.530, 0.559 respectively, in the order in which they are listed. These ratios stand in much the same relationship as the N₂/N₃ ratios reported by Javillier and Djelatides, and would be simpler to determine.

After observing these several ratios based upon protein fractionation as applied to the five flours reported in Table 6, Javillier and Djelatides applied the method to 32 additional flour samples of varying composition and qualities. These samples were also subjected to test by the Chopin

¹⁹ *Trab. IX Cong. Internac. Quimica Pura y Apl.*, VII, 1-10 (1934).

apparatus, and the physical constants thus determined, and discussed elsewhere by Bailey,²⁰ were compared with the nitrogen ratio. They observed that the flours of good quality as measured with the Chopin device had an N_2/N_3 ratio between 1 and 2, between 2 and 3 for medium-quality flours, and between 3 and 4 in the instance of poor-quality flours.

Table 6. Total Nitrogen (N.T.) and Nitrogen Fractions in Parts per Thousand of Flour as Reported by Javillier and Djelatides.¹⁹

	Farine de blé Sully	Farine de blé Providence	Farine de blé Aurèle Gaby	Farine de blé dur	Farine de blé Anna
N_1	355	250	375	395	350
N_2	540	720	725	1122	950
N_3	400	430	360	370	230
N_4	255	100	140	235	170
NT	1550	1600	1600	2120	1700
N_1 p. 100 NT	22.9	15.6	23.4	18.6	20.5
N_2 "	34.8	45.0	45.3	52.9	55.8
N_3 "	25.8	26.8	22.5	17.5	13.5
N_4 "	16.5	12.5	8.8	11.0	10.0
$N_2+N_3+N_4$	1195	1350	1225	1725	1350
N_2+N_3	940	1150	1080	1490	1180
$[N_2+N_3] \times 100$ $N_2+N_3+N_4$	78.6	85.1	88.1	86.3	87.4
$N_2 \times 100$ N_2+N_3	40.4	37.3	33.3	24.8	19.4
$N_3 \times 100$ $N_2+N_3+N_4$	33.4	31.8	29.3	21.4	17.0
N_2	1.35	1.67	2.00	3.02	4.10
N_3					
Quality of flour....	very good	good	good	poor	very poor

In a further study of the N_2/N_3 protein ratio, Geoffroy and Labour²¹ emphasized that this ratio must be interpreted in the light of the total nitrogen. Thus two flour samples, Vilmorin 27 (1935 crop) and Manitoba, had practically identical N_2/N_3 ratios, namely 1.35 and 1.37 respectively, but very different total nitrogen percentages, 1.60 per cent and 2.33 per cent. The latter was apparently reflected in the Chopin extensimeter tests, which were:

	W	G
Vilmorin 27	120	19.5
Manitoba	378	23.5

Methods for the Determination of Gliadin

Fleurent²² extracted the flour with 70 per cent ethanol containing 0.2-0.3 per cent KOH, the glutenin present being precipitated by passing CO_2 through the alkaline extract and recovering the precipitate by cen-

²⁰ "Wheat Studies of the Food Research Institute," 15 (6), 277 (1940).

²¹ *Bull. assoc. chim.*, 53, 1 (1936).

²² *Compt. rend.*, 123, 327 (1896).

trifuging. Gliadin was then determined in an aliquot of the supernatant liquid by drying at 105° C and subtracting the weight of K_2CO_3 present.

Osborne²³ apparently was satisfied with a procedure which involved direct extraction of the flour with aqueous ethanol solution (sp. gr. 0.90). although it should be noted that he was not primarily concerned with the quantitative estimation of gliadin content, but rather with the recovery of sufficient material to facilitate its further study.

Snyder²⁴ extracted 5 grams of flour with 250 cc of 70 per cent ethanol for 18 hours, filtered, and Kjeldahled 100 cc of the filtrate. He noted that with old or unsound flours a correction must be made for soluble amid bodies. Snyder²⁵ later described a polametric method which involved extracting 15.97 g of flour with 100 cc of 70 per cent ethanol at room temperature for several hours, filtering, and reading in a 220-mm tube in a saccharimeter. The reading on the sugar scale $\times 0.2$ was accepted as the equivalent of the gliadin nitrogen.

Teller²⁶ employed 75 per cent ethanol (presumably by volume) at a temperature just below the boiling point for extracting gliadin. The residue from the first extraction was re-extracted six times. Finally the nitrogen content of the *residue* was determined, and this was subtracted from the total nitrogen as previously determined, the difference being recorded as the gliadin nitrogen. The latter was multiplied by the factor 5.7 to give the percentage of gliadin. In a patent flour thus analyzed the gliadin constituted 64 per cent of the total nitrogen.

Later²⁷ Teller extracted flour with a series of aqueous ethanol solutions ranging in concentration from 40 to 95 per cent of alcohol, and found that the lower concentrations from 40 to 60 per cent extracted the most nitrogenous material. Also treatment of flour with 95 per cent ethanol (which extracted relatively little protein itself) prevented the subsequent extraction of protein from the residue with 75 per cent ethanol. Teller adopted the use of ethanol (sp. gr. 0.90) as the gliadin solvent.

Chamberlain,²⁸ as referee of the Association of Official Agricultural Chemists in 1904, subjected Snyder's polarimetric method to collaborative study in comparison with a direct alcoholic extraction followed by Kjeldahling the extract. The findings of the collaborator (H. Snyder) by the two methods were in good agreement, but a second collaborator reported much lower results by the polarimetric method. Chamberlain recommended that no names be applied to the protein materials separated by the various extraction methods, but that they be described only in

²³ "The Proteins of the Wheat Kernel," Carnegie Institution of Washington, 1907.

²⁴ *Minn. Agr. Exp. Sta. Bull.* 65, p. 519 (1899).

²⁵ *J. Am. Chem. Soc.*, 26, 263 (1904).

²⁶ *Ark. Agr. Exp. Sta. Bull.* 42, p. 85 (1896).

²⁷ *Ark. Agr. Exp. Sta. Bull.* 53, p. 63 (1898).

²⁸ *U. S. Dept. Agr., Bur. Chem. Bull.* 90, p. 121 (1905).

terms of the process of separation. Proteins soluble in alcohol were to be determined provisionally by extracting 2 grams of flour with 100 cc of 70 per cent ethanol for 18 hours (following a period of 15 minutes of continuous shaking), filtering into a Kjeldahl flask, and washing the residue with 100 cc of 70 per cent alcohol. The combined extracts were then to be Kjeldahled, and the percentage of nitrogen multiplied by the factor 5.68.

Hot 70 per cent ethanol extracted less protein from flour than cold alcohol in Chamberlain's²⁹ experiments. This is contrary to the usual experience, and Chamberlain accounted for it by assuming that the hot solution had a coagulating action. When a flour was extracted directly with 70 per cent ethanol 7.47 per cent of protein ($N \times 5.7$) was recovered, but when the same flour was first extracted with salt solution and the residue was extracted with 70 per cent ethanol, only 5.50 per cent of protein was recovered in the alcohol solution. In terms of percentage of total proteins, the following proportion of protein was recovered by several extractions as described:

Direct extraction with alcohol	56.73%
Direct extraction with salt solution	20.80
Extraction with alcohol after preceding extraction with salt solution ...	41.20
Extraction with salt solution after preceding extraction with alcohol ...	4.92
Extraction of the salt solution extract with alcohol	12.70

Accordingly, Chamberlain expressed doubt concerning the justifiability of attempting a separation of the wheat proteins on the basis of solubility, "because of the indefinite value of such separation."

The quantity of protein material extracted by ethanol solutions of varying concentration, as determined by Kjeldahl³⁰ had a minimum at 20 per cent ethanol, a maximum at 55 per cent, and neared zero at 90 per cent. In 55 per cent ethanol solution the specific rotatory power of the protein was -92° ; in glacial acetic acid -82° ; in dilute acetic acid (5 per cent and less), -111° ; in dilute alkali about the same; in phenol (α)_D^{40°} = -130° .

The earlier work on the determination of gliadin was reviewed by Snyder,³¹ who reported on the amount of protein dissolved by aqueous ethanol solutions of varying concentrations. The resulting data, recalculated by the present author as percentage of the maximum extracted, are recorded in Table 7.

Snyder then recommended that 70 per cent ethanol by weight, specific gravity 0.871, be employed for the extraction of alcohol-soluble proteins. This is equivalent to a concentration of somewhat more than 77 per cent

²⁹ *J. Am. Chem. Soc.*, 28, 1657 (1906).

³⁰ *Biedermanns Zent.*, 25, 197 (1896).

³¹ *U. S. Dept. Agr., Bur. Chem. Bull.* 105, p. 88 (1907).

Table 7. Proportion of Protein Extracted by Aqueous Ethanol Solutions of Varying Concentration, Recorded in Terms of the Maximum Amount Extracted, from Snyder's³¹ report.

Ethanol solvent		Proportion extracted in terms of the maximum	
Ethanol by weight (%)	Ethanol by volume (%)	Snyder	Shutt
60	67	100	100
62.5	69.5	—	99
65	72	—	98
68	75	87	—
70	77	82	83
72	79	79	—
75	81	—	64
76.8	83	—	70
86.4	90	—	13

by volume, which is decidedly more concentrated than the solvent used chiefly by Osborne.

The following year Snyder³² commented that "there is such an extensive overlapping of the proteids as to solubility that it is doubtful whether accurate analytical methods can be devised for the determination of any one proteid." He again emphasized the necessity for designating the products as "water-soluble," "salt-soluble," or "alcohol-soluble," rather than by specific names.

Gliadin was extracted from flour by Thatcher³³ with 70 per cent ethanol by weight, and the factor $N \times 6.25$ was applied, although it was admitted that this factor was too high. As *gliadin number*, Thatcher expressed the proportion of gliadin in the total protein of flour. In the 26 samples analyzed by Thatcher the gliadin number ranged from 32.5 per cent in a low-protein (8.70 per cent) Fife wheat flour to 55.5 per cent in a Little Club wheat flour.

A method was suggested by Ladd³⁴ which involved the extraction of 4 grams of flour with 200 cc of alcohol (sp. gr. 0.90) for 15 hours, filtering, and determining the protein by Kjeldahling in 100 cc of the filtrate. The nitrogen minus the amid nitrogen is taken as the alcohol-soluble proteid nitrogen, or gliadin. While Ladd does not give the factor in this section to be used in converting nitrogen content into terms of protein, it must be assumed that he intended $N \times 6.25$ to be used, since that is the factor recommended for total, globulin and albumin, and glutenin estimation.

Harcourt's³⁵ suggestion two years later was not materially different. It specified 70 per cent ethanol by volume as the solvent, an extraction period of 21 hours, filtration, and the Kjeldahling of an aliquot. No factor was recommended for converting the percentage of nitrogen into terms of protein.

³² U. S. Dept. Agr., Bur. Chem. Bull. 116, p. 52 (1908).

³³ J. Am. Chem. Soc., 29, 910 (1907).

³⁴ U. S. Dept. Agr., Bur. Chem. Bull. 122, p. 55 (1909).

³⁵ U. S. Dept. Agr., Bur. Chem. Bull. 137, p. 149 (1911).

A rather exhaustive study of the extraction of proteins from flour with ethanol solutions was conducted by Hoagland,³⁶ which included period of extraction, temperature, and concentration of ethanol. As in the instance of Teller's studies, the percentage of protein extracted passed through a maximum when alcohol of 50 per cent concentration by weight (about 57 per cent by volume) was used as the solvent. Elevating the temperature to 75° C increased the quantity of protein extracted by about one-third. Hoagland concluded that 50 per cent alcohol was the logical solvent, and that 60 to 90 minutes of continuous shaking is as satisfactory as a longer period of extraction with less vigorous agitation throughout, as in the older techniques.

Concurrently with these studies, Greaves³⁷ was engaged in an even more elaborate study of the quantitative determination of gliadin. As in the earlier studies, the quantity of protein extracted from flour by more concentrated ethanol, *viz.*, 80 per cent by volume, was definitely less than was extracted by 60-70 per cent ethanol by volume. When the extraction was conducted at 65° C, more protein was extracted than at room temperature, but the specific rotation of the protein extracted indicated the presence of more non-gliadin protein material at the higher extraction temperature. On the basis of his researches Greaves recommended that alcohol of such a concentration should be used as the gliadin solvent that, with the moisture in the flour, it should be equivalent to 74 per cent by volume. The flour suspension should be shaken occasionally for 24 hours, allowed to settle 24 hours, and then filtered through a ½-inch asbestos pad in a Gooch crucible. An aliquot of the filtrate may then be Kjeldahled.

The percentage of the total protein in a high protein (17.31 per cent dry matter basis) flour and a low protein (11.11 per cent dmb.) flour that was extracted by several solvents was reported by Schleimer.³⁸ With methanol the maximum proportion of the protein was extracted with 55-60 per cent aqueous solutions, the proportion being from 51.3 to 51.6 per cent of the total flour. Ethanol extracted more protein than methanol, the maximum proportion, 59.1 to 59.5 per cent of the total protein being extracted with 55 per cent aqueous ethanol solutions. Propanol extracted the largest proportion of the total protein, namely 62.6 to 64.7 per cent, at a concentration of 40 per cent propanol in aqueous solution.

Only part of the alcohol-soluble protein is really gliadin, in the opinion of Olson,³⁹ the average being about 68 per cent. His estimate is based upon the percentage of gliadin which precipitates when the alcohol present in the original extract is evaporated by heat and replaced with water.

³⁶ *J. Ind. Eng. Chem.*, **3**, 838 (1911).

³⁷ *Univ. Calif. Pubs. in Physiol.*, **4**, 31 (1911).

³⁸ *Z. ges. Getreidew.*, **3**, 138 (1911).

³⁹ *J. Ind. Eng. Chem.*, **5**, 917 (1913).

Since the highest percentage of coagulable nitrogen was encountered in the extracts prepared with 50 to 65 per cent alcohol by volume, Olson adopted 50 per cent alcohol as the gliadin solvent. As a matter of convenience, he determined the nitrogen content of the extract (I), and of the filtrate resulting from coagulating the gliadin by heat followed by evaporation of the alcohol (II). The difference between I and II was taken as the gliadin nitrogen of the sample.

The solubility of wheat proteins in ethanol of various concentrations was also studied by Fanto,⁴⁰ who likewise observed a low solubility in 20 per cent ethanol, rising to a maximum at 50+ per cent, and approaching zero at concentrations above 90 per cent.

An attempt to estimate the relative purity of alcoholic extracts of flour in terms of gliadin, by hydrolyzing the proteins in the extracts and determining the ammonia in the hydrolyzates was made by Bailey and Blish.⁴¹ Since gliadin yielded 25.5 per cent of its nitrogen as ammonia, whereas albumin and globulin yielded 6.8 and 7.7 per cent respectively, the proportion of gliadin in mixture with the non-gluten proteins was assumed to be reflected in the ammonia content of the hydrolyzate. On the basis of this assumption it appeared that the relative purity in terms of the gliadin of protein mixtures extracted with 50 per cent ethanol by volume was as great as that extracted with 70 per cent ethanol, the gliadin constituting 92.9 and 92.6 per cent respectively of the two extracts. Since the 50 per cent ethanol extracted more total protein than the 70 per cent ethanol, it appeared logical to employ the former as the gliadin solvent. When 50 per cent ethanol was used at the boiling temperature, *viz.*, 83°-84° C, more protein of the same purity appeared to be extracted than when the extraction was conducted at room temperature. Reference will be made later to the observations of Sharp and Herrington and of Blish and Sandstedt respecting the use of hot alcohol in this connection.

From these experiences with ethanol of various concentrations as a solvent for prolamins, the author concludes that such proteins, in order to be peptized by ethanol, must first be hydrated to a certain minimal value. If the concentration of alcohol is too high, incipient denaturation of the protein occurs by withdrawal of the water of hydration into the alcohol. When the concentration of water is too high, the strongly hydrated gliadin loses its capacity to disperse in the alcoholic medium. The gradation in "solubility" observed on progressively increasing the concentration of alcohol may be due either to: (1) the presence of numerous prolamins in the mixture, each of which behaves differently in these

⁴⁰ *Z. Untersuch. Lebensm.*, **24**, 269 (1912).

⁴¹ *J. Biol. Chem.*, **23**, 345 (1915).

respects, or (2) to the complexity of the gliadin molecule itself, with varied "hydration centers," each of which exhibits a distinctive hydration capacity. If the distribution of the water to these centers is not uniform, certain molecules may acquire sufficient of it to be dispersed by alcohol, while other molecules remain closely associated with like molecules in an "undissolved" matrix.

The various protein fractions of flour were studied by Sharp and Gortner,⁴² including the protein directly extracted by 70 per cent ethanol (A.O.A.C. method) (I), and that extracted by ethanol following a previous extraction with 5 per cent K_2SO_4 solution (II). Fraction II contained less protein based upon the original flour than fraction I. Thus the average of II in the instance of eleven flours of widely varying protein content was about 74 per cent of the average of I. Apparently Sharp and Gortner were inclined to accept II as a more accurate measure of the alcohol-soluble fraction; at least, when it was combined with the percentage of the protein extracted with 5 per cent K_2SO_4 solution, the difference between this sum and the total protein content was taken as representing the glutenin content of these flours, to avoid cumulative errors.

Certain phases of these and related studies on the protein fractions resulting from a series of successive extractions of flour with various solvents were re-investigated by Herd.⁴³ He found that the proportion of the total protein appearing in an alcoholic extract following a pre-extraction with a saline solution depended upon the nature of the salt solution first applied. This suggested that "gliadin" is not a distinct entity.

Sharp and Herrington⁴⁴ employed the same technique as a standard practice. They also observed that more protein was extracted by an alcohol 50 to 60 per cent by volume than by any other concentration, thus confirming the earlier work. This phase of their study was amplified by reflexing at the boiling temperature, and it was found that boiling 50 per cent alcohol extracted the most protein. Moreover, the boiling alcohol at concentrations from 50 to 70 per cent extracted about 20 per cent more protein than alcohol at room temperature.

In wheat-rye hybrids analyzed by Kretowitch,⁴⁵ the percentage of the total protein extractable by 70 per cent ethanol appeared to be less than in wheat. Thus the "gliadin-N" fraction ranged from 41.9 to 48.1 per cent, averaging 45.7 per cent in a collection of four hybrids furnished by Meisters of the Saratower (U.S.S.R.) Station, whereas the winter wheat used for comparison yielded 52.6 per cent of its nitrogen to the ethanol extract. Incidentally, it was reported that three of these rye

⁴² *Minn. Agr. Exp. Sta. Tech. Bull.* 19 (1923).

⁴³ *Cereal Chem.*, 8, 1 (1931).

⁴⁴ *Cereal Chem.*, 4, 249 (1927).

⁴⁵ *Planta*, 25, 64 (1936).

hybrids were of later generations, which bore a close resemblance to wheat.

On heating wheat for 3 and for 6 hours at 130° Kretovič and Riazanceva⁴⁶ found the flour "gliadin," or protein soluble in 70 per cent ethanol, to be reduced to 48.5 and 44.5 per cent respectively in terms of the total protein, contrasting with 58.0 per cent in the control or unheated wheat. This was in an experiment with Manitoba wheat. Using a North Caucasian wheat, var. "Erythro-spermum," the control wheat flour yielded 41.59 per cent of its total protein to alcohol extraction, whereas on heat-treating, as described above, the "gliadin" return was reduced to 30.3 and 24.5 per cent respectively. Less vigorous heat treatments had correspondingly smaller effects.

The tentative method of the Association of Official Agricultural Chemists appearing on page 217 of their latest Book of Methods (fifth edition, 1940) for 70 per cent alcohol-soluble proteins is essentially as follows:

4 g of flour is extracted with 100 ml of ethanol, 70 per cent by volume, with vigorous shaking at 30-minute intervals over 5 or 6 hours (or continuous shaking for 1 hour in a shaking machine), followed by undisturbed extraction overnight. The next day the preparation is again shaken, and then filtered clear. A 50-ml aliquot is Kjeldahled, and the N then determined is multiplied by the factor 5.7 to convert into the equivalent percentage of protein. It is significant that the term "gliadin" is not employed in connection with this determination.

Another tentative method by polarization is also described in the same A.O.A.C. Book of Methods which involves extracting 15.97 g of flour with 100 ml of ethanol (sp. gr. 0.90*). The extraction treatment is essentially as described above. Following filtration, the extract is observed in a 200-mm. saccharimeter tube, the readings being recorded in the Ventzke scale. The proteins are then precipitated in an aliquot by means of Millon's reagent, and filtered off. The resulting solution is again observed in the saccharimeter and the reading multiplied by 1.1 to correct for dilution with the Millon's reagent. The second reading is deducted from the first and the difference, multiplied by 0.2, is the percentage of gliadin nitrogen.

The protein methods recommended in the manual of methods for cereal laboratories recently published by the American Association of Cereal Chemists do not differ materially from those described in the A.O.A.C. Book of Methods.

* About 66 per cent by volume.

⁴⁶ *Compt. rend. acad. sci. U.S.S.R.*, 3, 409 (1935).

Preparation of Gliadin

Dill and Alsberg^{47, 48} improved upon the earlier methods of isolating and purifying gliadin. Their concentrated syrup resulting from evaporating the alcoholic extract at low pressure was poured into 5 volumes of 1 per cent NaCl solution. On vigorous shaking the gliadin precipitated as a foam which could be recovered, rewashed with more saline solution, and then dissolved in a solution which contained 70 per cent of alcohol by volume. On cooling in the refrigerator, some of the gliadin separated as a honey-like layer, from which clear (at room temperature) gliadin solution could be decanted. The residue was treated with warm alcohol, again cooled, and the undissolved residue, containing much of the lipoids and suspensoid impurities, was rejected. The two decantates were combined, concentrated at low pressure and the gliadin precipitated in LiCl solution. The wet gliadin was then dissolved in a minimum quantity of strong alcohol, reprecipitated by pouring slowly into absolute alcohol containing 0.025 per cent LiCl. This was repeated a third time, the precipitate then dehydrated with absolute alcohol, and dry aldehyde-free ether, and finally dried at 40° C and 20 mm pressure.

Of course, this procedure is not qualitative, since there is a loss of prolamins in the residues formed on cooling. Dill and Alsberg felt that an unusual degree of purity was attained, however. They call attention to the fact that denaturation of gliadin may occur on standing in contact with 75 to 85 per cent alcohol; and the denatured protein will not dissolve subsequently in any concentration of alcohol at room temperature.

Their preparations contained an average of 17.54 per cent nitrogen, which is the same as that of the Mathewson, Groh and Friedl, Cross and Swain, and Tague preparations, and only slightly lower than Osborne and Voorhees' preparation, which contained 17.66 per cent.

Dill and Alsberg also observed the "critical peptization temperature" of gliadin solutions, *i.e.*, the temperature at which solutions prepared by saturating the alcohol solvent at 50° C first became turbid on cooling. The turbidity temperature was an inverse function of the quantity of gliadin per unit of solvent. At 2.5 g per 100 g of alcohol solution, it was 70° C in 50 per cent ethanol, 2° C in 60 per cent ethanol, and 6° C in 70 per cent ethanol. A series of curves is presented involving concentration of *N*-propyl, ethyl, and methyl alcohols as variables, and the critical peptization temperature was as low as -6.5° C. with 50 per cent *n*-propyl alcohol, 2° C in 60 per cent ethyl alcohol, and 10.5° C in 65 per cent methyl alcohol, when 2.5 per cent solutions of gliadin were used. In gen-

⁴⁷ *J. Biol. Chem.*, **63**, Proc. lxvii (1925).

⁴⁸ *J. Biol. Chem.*, **65**, 279 (1925).

eral the peptizing temperature range is greatest with *n*-propyl, second with ethyl, and lowest with methyl alcohol. These authors comment that evidently there is no upper limit to the solubility of gliadin in suitable alcohol-water mixtures above the critical solution temperature curve. This suggests that a system of three liquids is involved, two of which, water and alcohol, are miscible in all proportions. The third, solvated gliadin, is completely miscible with the other two above the critical peptization temperatures. Apparently both water and alcohol are adsorbed, since gliadin is not dissolved or peptized by either solvent when pure.

In this general connection Dill and Alsberg failed to effect a ready diffusion of gliadin (in 60 per cent alcohol) through vegetable parchment or collodion.

The molar concentration of various substances capable of holding gliadin in solution at 20° C as determined by dilution was also determined by Dill and Alsberg, with the following results: potassium thiocyanate 1.06*M*; ammonium thiocyanate 1.09*M*; potassium iodide 5*M* (foaming rendered the value uncertain); urea 2.03*M*; methyl alcohol 12.1*M*; ethyl alcohol 5.8*M*; *n*-propyl alcohol 2.28*M*; isopropyl alcohol 4.17*M*; choral hydrate 3.0*M*.

Glycerol, ethylene glycol, each of the propylene glycols, mixtures of water with each of these, mixtures of glycerol and absolute alcohol containing in excess of 25 per cent glycerol, and mixtures of water with methyl ethyl ketone containing from 10 to 25 per cent by volume of the latter gave clear solutions of gliadin at room temperature. Saturated aqueous solutions of *n*-butyl alcohol, ammonium nitrate, and ammonium chloride did not seem to peptize gliadin, nor did NH_3 .

Gottenburg and Alsberg⁴⁹ further observed that prolonged contact of gliadin with 20 and 30 volume per cent, and 80 volume per cent ethanol alters its solubility, but at intermediate concentrations very little alteration occurs even at higher temperatures.

Blish and Sandstedt⁵⁰ found that if crude gluten was dried *in vacuo* at 65-70° C, ground to a powder, and treated with 0.07*N* acetic acid, only gliadin was dispersed. It could then be recovered by the addition of 7 + g of lithium chloride per liter, when it promptly precipitated. This moist preparation would readily dissolve in 60 per cent by volume ethyl alcohol. Reprecipitation with lithium chloride resulted in gliadin preparations of high purity as indicated by nitrogen content, ammonia in the hydrolyzate, specific rotatory power and freedom from carbohydrates.

Proteins extracted from flour with hot ethanol were studied by Blish and Sandstedt,⁵¹ who concluded that at the boiling temperature some of

⁴⁹ *J. Biol. Chem.*, **73**, 581 (1927).

⁵⁰ *Cereal Chem.*, **3**, 144 (1926).

⁵¹ *Cereal Chem.*, **6**, 494 (1929).

the glutenin is dispersed. The peptized portion appears to have a different constitution from native glutenin to the extent that it yields more ammonia on hydrolysis. This was established by treating a purified glutenin preparation with boiling 70 per cent alcohol, about 10 per cent of the preparation being peptized on the first treatment, and 6 per cent on the second treatment. Moreover, the first extract yielded 16.90 per cent of its nitrogen as ammonia on hydrolysis, while the combined extracts Nos. 2 to 5 yielded 20.95 per cent, as against only 12.4 per cent in the instance of the original glutenin preparation.

Eto⁵² attempted to determine the "solubility" of gliadin in various solvents. In ethanol of various concentrations the results were as follows:

90% ethanol	0.39% of dissolved gliadin (at 30° C)
80% "	1.06% " "
70% "	unlimited
60% "	"
50% "	1.12% of dissolved gliadin
40% "	0.54% " " "

In a series of aqueous HCl and NaOH solutions of progressively increasing concentration, ranging from *N*/5120 to *N*/2, the solubility of gliadin appeared to pass through a maximum in the range of *N*/320 HCl, and *N*/40 NaOH. With pH as the observed variable in a mixture of HCl and NaOH, the solubility was higher at pH 4.1 than at higher levels, although it increased on passing from pH 9.9 to 10.3.

An isoelectric point of pH 6.6 was observed when the quantity of gliadin precipitated was used as the criterion.

The solubility of gliadin in different solvents was also studied by Tague,⁵³ using a gliadin preparation made according to the method described by Osborne.⁵⁴ In each measurement of solubility, an excess of gliadin was added to the solvent, filtered after standing overnight, and an aliquot of the filtrate was Kjeldahled to determine the proportion or quantity dissolved. Included in the findings were the following: 0.01*N* HCl dissolved more gliadin (140 mgm per 100 cc) than other concentrations of HCl ranging from 1.0 to 0.00001*N*. With H₂SO₄ the greatest solubility was encountered at 0.001*N* (126 mgm) and with acetic acid at 1.0*N* (173 mgm). The "solubility" was not a definite, clear-cut value, such as is encountered with simple salts, however, but varied with the ratio of gliadin to solvent, and increased progressively with time up to 10 days.

With dry gliadin, the apparent solubility increased with time up to 240 hours when it reached 80 mgm per 100 cc. When a moist gliadin

⁵² *J. Biochem. (Japan)*, 3, 373 (1924).

⁵³ *Cereal Chem.*, 2, 117 (1925).

⁵⁴ "The Proteins of the Wheat Kernel." Carnegie Institution of Washington, 1907.

preparation was used, the "solubility" was greater throughout, but again it increased progressively with time, reaching 163 mgm after 240 hours.

Tague also employed a series of solutions of chlorides and sulfates of the alkali, and alkaline-earth metals. In general, the solubility of gliadin was low, and tended to increase with increasing dilution. This was not true with Na_2SO_4 and MgCl_2 solutions, however. With the former, the maximum solubility was in 0.05*M*, when it reached 11.4 mgm per 100 cc and then decreased to 2.9 mgm at 0.0001*M*. With MgCl_2 solutions the behavior was peculiar, since a saturated solution dissolved 84 mgm per 100 cc, and the solubility then decreased regularly with increasing dilution, reaching 3 mgm at 0.0001*M*.

Using aqueous methanol solutions, Tague found them to be excellent solvents, the maximum solubility (132-133 mgm per 100 cc) being reached at concentrations of 60 to 70 per cent methanol by volume.

In these studies Tague noted that the method of preparation, *i.e.*, the pre-treatment to which the gliadin had been subjected, had a profound effect upon its subsequent solubility, a characteristic to which further reference will be made in reviewing the work of Sinclair and Gortner.

Sinclair and Gortner⁵⁵ extended the earlier studies made in the latter's laboratory on the peptization of flour proteins, giving special attention to gliadin. They soon found that gliadin preparations (A) made by recovery from alcoholic extracts would not completely redissolve in alcohol after the preparations had been dried. When heated, more of the gliadin passes into "solution," but, on cooling, a part of the gliadin again separates, giving a turbid solution. On the other hand, gliadin (B) prepared by the acetic acid method of Blish and Sandstedt⁵⁶ proved readily and completely soluble in 70 per cent ethanol, and did not precipitate or become turbid on long standing. It was also soluble in distilled water, while the gradual addition of gliadin to this dispersate resulted in a progressive dispersion until the preparation became very viscous. Such aqueous "solutions" were more acid (pH 3.99) than equivalent solutions of the gliadin extracted with alcohol (pH 6.46), and it required nearly three times as much standard alkali to neutralize a unit quantity to phenolphthalein. This suggests that the adsorption of traces of acetic acid by the protein is probably responsible for the solubility behavior of the preparation (B). When (B) was electrodialyzed this union with the acetic acid apparently was broken, and an aqueous solution of this preparation (D) had more nearly the same degree of acidity (pH 5.14) as preparation (A).

The gliadin preparations were also peptized by neutral salt solutions,

⁵⁵ *Cereal Chem.*, 10, 171 (1933).

⁵⁶ *Cereal Chem.*, 3, 144 (1926).

KI solutions being more effective than KBr solutions, and KCl the least of this series. This "solubility" was not a precise or definite property, however. Thus if the portion of gliadin which failed to dissolve initially in molar KI solution (F) was manipulated by repeated reprecipitation from ethanol, the resulting preparation was then capable of being further peptized by molar KI solution. A study of the nitrogen distribution after acid hydrolysis of these several gliadin preparations, using the Hausmann method, failed to disclose any differences between them. Sinclair and Gortner contend that the data of Haugaard and Johnson⁶¹ do not afford evidence of any differences in the chemical constitution of the latter's gliadin fractions, since the differences are within the range of experimental error of the methods employed. These peptization behaviors appear, rather, to reflect the physical state of the protein gel, which in turn may be the consequence of the last treatment accorded the preparation.

The fractionation of gluten by precipitation from urea solutions at different salt concentrations, as practiced by Cook,⁵⁷ showed no clear-cut distinction between the solubilities of gliadin and glutenin in this mixed solution. When the concentration of MgSO_4 in terms of saturation was 0.13, 0.16, 0.18, 0.21, 0.27, and $0.5 \pm$, the percentage of gliadin in the precipitate was 18, 32, 50, 70, and 80 respectively, the remainder being assumed to be glutenin. The arginine content of the precipitated protein decreased fairly regularly with increasing concentration of MgSO_4 as the precipitant, ranging from 8.2 per cent of the protein precipitated at 0.13 saturation to 5.8 per cent in the protein precipitated at $0.5 \pm$ saturation with MgSO_4 .

The concentration of MgSO_4 required to precipitate gliadin from its solution in 30 per cent urea, as a function of the concentration of dissolved gliadin, was noted by Cook and Alsberg.⁵⁸ With from 1 to 10 per cent of gliadin in the solution the equivalent of 13 to 15 per cent of saturation with MgSO_4 produced a permanent turbidity, while 14 to 18 per cent of saturation effected a definite slight precipitation. While the differences were not large, there appeared to be a trend in the direction of a larger dosage of MgSO_4 required to produce these effects as the concentration of gliadin was reduced.

Cook⁵⁹ made five gliadin preparations, after the methods (I) of Osborne, (II) of Dill and Alsberg, (III and IV) of Blish and Sandstedt, and (V) urea dispersion. The last method involved dispersing crude gluten in 30 per cent urea, removing starch in the supercentrifuge, and

⁵⁷ *Can. J. Research*, **5**, 389 (1931).

⁵⁸ *Can. J. Research*, **5**, 355 (1931).

⁵⁹ *Can. J. Research*, **5**, 389 (1931).

precipitating the glutenin at 0.18 of saturation with MgSO_4 . After removing the glutenin by supercentrifuging, 250 g of solid MgSO_4 was added to each liter of the liquid which remained. The resulting precipitate was recovered in a supercentrifuge. Although the liquid removed at this stage contained protein, it was discarded. The precipitate was redispersed in 30 per cent urea solution, MgSO_4 equivalent to 0.12 saturation was added, and most of the protein precipitated. On redispersing in 10 per cent urea and passing through the supercentrifuge, a clear solution was obtained. This was poured into five volumes of water, and allowed to settle for 24 hours. The precipitate was removed, washed, and dehydrated with alcohol and ether. The resulting gliadin preparation was dispersed by 60 per cent alcohol, but the dispersion was more opaque than that of gliadin prepared by the alcohol method.

Although the physical properties of these five gliadin preparations varied considerably in terms of viscosity and critical precipitation temperatures, their nitrogen content, Hausmann numbers, and arginine content were very similar. The nitrogen content ranged between 17.24 and 17.71 per cent, and the extremes were represented by the two preparations made by the Blish and Sandstedt methods.

In a study of the effect of alkali on gliadin, α -glutenin, gluten (and casein) Csonka and Horn⁶⁰ were convinced that in 0.5 and 0.5M NaOH solutions these proteins were disintegrated even at 21° C. At 38° the hydrolytic process proceeded much more rapidly. Accordingly, they suggested that the term "racemized protein" for these alkali digestion products be discontinued. They contend that Dakin's theory of keto-enol formation does not afford a satisfactory explanation for their observations.

Haugaard and Johnson⁶¹ noted that solutions of gliadin in ethanol prepared at room temperatures tended to separate partially on cooling the solution. In their second and most involved study, 600 g of purified gliadin was dissolved in sufficient 60 per cent (by vol.) ethanol to give a 10 per cent solution of gliadin. This was allowed to stand at 0° C for several days, when the precipitate that appeared was recovered, washed with absolute ethanol, ether, and then dried. This was fraction IV. The clear solution was then brought to -11° C, when another precipitate formed which was recovered as in the first instance, and labeled fraction II. The original unfractionated gliadin solution was called fraction III, while fraction I was recovered from the clear solution which remained after holding the solution at -11° C. They obtained 15 g of I, 280 g each of II and IV, and 200 g of III. The nitrogen content of the four frac-

⁶⁰ *J. Biol. Chem.*, **93**, 677 (1931).

⁶¹ *Compt. rend. trav. lab. Carlsberg*, **18**, 1 (1930).

tions was practically the same, averaging 17.65 per cent. Their acid and base binding capacity was the same for fractions II, III and IV, and the isoelectric reaction in 52.2 per cent alcohol (by weight) was calculated to lie at about pH 7.1. No substantial differences were observed in the products of acid hydrolysis of the fractions. The largest differences were found in the tryptophane and tyrosine contents. Thus the most readily soluble fractions (I and II) had the highest tryptophane and the lowest tyrosine content. Apparently the only marked differences between these thermal fractions discerned by Haugaard and Johnson were in the physical properties of optical rotation and viscosity. Thus at equivalent concentrations fraction IV gave the most viscous solutions in 60 per cent ethanol by volume, followed by III, II, and I in the order named. The specific equivalent rotation in the same solvent, at different levels of pH , and observed at three wave lengths of light, was in the same order, namely IV greatest, followed by III and II. No such measurement was made with fraction I.

The relative solubility of the proteins in wheat of three genetic species, *T. monococcum*, *T. vulgare*, and *T. durum*, was determined by Kniaginicev and Gorelkina,⁶² who concluded that the protein complex varies from species to species. The 70 per cent ethanol-soluble nitrogen (in per cent of the total N) was 7.5, 11.8 and 17.7 per cent respectively in samples of the three species in the order named. (Note by the author: These values are very low as contrasted with the data resulting from the application of conventional procedures, and the brief and wholly inadequate description of the techniques employed that are included in the English report sent to me does not afford a basis for interpreting the findings.)

Both the gliadin and the glutenin prepared from *Triticum polonicum*, or Polish wheat, were observed by Blish and Pinckney⁶³ to be less levorotatory than analogous preparations made from *T. vulgare* varieties Kanred and Marquis. Their data are recorded in Table 8.

No difference in the isoelectric range as determined by flocculation methods in gliadins prepared from Japanese, American and Australian wheats was observed by Kondo and Hayashi,⁶⁴ and, on this basis, they concluded that the several gliadins were identical in character.

Changes in viscosity of gliadin solutions as a function of the relative normality of the several acids that were employed in adjusting the degree of acidity were determined by Luers.⁶⁵ The degree of acidity was not determined and recorded as pH , however, and, accordingly, it is possible only to approximate an estimate of the latter, since the degree of buffer-

⁶² *Compt. rend. acad. sci. U.R.S.S.*, 16, 419 (1937).

⁶³ *Cereal Chem.*, 1, 309 (1924).

⁶⁴ *Mem. Coll. Agr. Kyoto Imp. Univ.*, 11, 21 (1931).

⁶⁵ *Kolloid-Z.*, 25, 177 (1919).

ing of these solutions by the protein itself and by other substances which may have been present is not known. With lactic acid the non-acidulated solution was turbid; the turbidity gave place to an opalescent solution of increasing viscosity upon the progressive addition of acid until the normality was equivalent to 1.8×10^{-2} . Further additions of acid, or increase in normality, resulted in diminishing viscosity. The maximum viscosity was reached at the following levels of normality in the instance of other acids and alkalis:

sulfuric acid	2.2×10^{-3}
hydrochloric acid (in alc. soln.)	1.5×10^{-3}
sodium hydroxide	2.2×10^{-3}
barium hydroxide	$1.3-1.8 \times 10^{-3}$

Specific Rotatory Power of Gliadin

It was early observed that gliadin is optically active in alcoholic solutions, and Kjeldahl⁶⁶ in 1892 reported its specific rotatory power to be -92° in 55 per cent ethanol. Snyder⁶⁷ proposed a polarimetric method for the quantitative estimation of gliadin. This involved extracting 15.97 g of flour with 100 cc of 70 per cent ethanol at $20^\circ \pm$ for several hours, filtering, and reading in a 220-mm tube in a saccharimeter. The reading on the sugar scale was multiplied by 0.2 to give the equivalent of *gliadin nitrogen*. Snyder did not correct for the sugars present, since he apparently assumed a constant error from that source. He mentioned that gliadin can be precipitated from such solutions by mercuric nitrate, and the correction noted after thus removing the proteins "should extreme accuracy be necessary." This was a practice in which later investigators not uncommonly indulged.

In the collaborative studies reported by Chamberlain⁶⁸ this method was compared with the results obtained by Kjeldahling the alcoholic extracts (factor $N \times 5.68$). In the hands of Snyder, as one of the collaborators, the polarimetric method gave results practically identical with the Kjeldahled extracts, but Ladd and Kimberley's results were uniformly lower by the order of about 30 per cent when the polarimetric method was followed. It appears that the polarimetric method was subsequently employed very little as a quantitative procedure, although Greaves⁶⁹ contended that such determinations can be made rapidly and the results are fairly accurate. He preferred to use half the charge of flour proposed by Snyder, and suggested that it may be advisable to read

⁶⁶ Forhandl. Skand. Naturfors., p. 385 (1892). Abst. in J. Chem. Soc. 77, 583 (1896). Original not seen.

⁶⁷ J. Am. Chem. Soc., 26, 263 (1904).

⁶⁸ U. S. Dept. Agr., Bur. Chem. Bull. 90, 121 (1905).

⁶⁹ Univ. Calif. Pub. Physiol., 4, 31 (1911).

Table 8. Specific Rotatory Power $[\alpha_D]$ of Gliadin.

Investigator	Solvent	(α) _D
Kjeldahl ⁷⁰	0.1% — 5% acetic acid	—110.0°
"	55% ethanol	— 92°
"	40% phenol	—130.0°
"	anh. acetic	— 81.0°
Osborne and Harris ⁷¹	80% ethanol	— 92.55°/20°
"	"	— 91.9°/20°
Mathewson ⁷²	75% ethanol	— 88.2°/20°
"	70% ethanol	— 93.1°/20°
"	70% ethanol	— 92.1°/20°
"	70% ethanol	— 91.8°/20°
"	70% ethanol	— 92.9°/30°
"	70% ethanol	— 91.3°/40°
"	60% ethanol	— 96.9°/40°
"	60% ethanol	— 96.4°/40°
"	50% ethanol	— 98.6°/40°
"	50% ethanol	— 98.3°/40°
"	65% propanol	—101.1°
"	70% phenol	—123.1°
"	anh. phenol	—131.6°
"	para cresol	—121.0°
"	benzyl alcohol	— 55.7°
"	anh. acetic acid	— 79.8°
Kossel and Kutscher ⁷³	70% ethanol	— 92.0°
Lindet and Ammann ⁷⁴	70% ethanol	$\alpha =$ — 81.6°
"	70% ethanol	$\beta =$ — 95.0°
Greaves ⁶⁹	60% ethanol	— 80.85°
"	65% ethanol	— 86.52°
"	70% ethanol	— 79.49°
"	72.5% ethanol	— 87.46°
"	74% ethanol	— 89.80°
"	75% ethanol	— 89.16°
"	80% ethanol	— 89.71°
Groh and Friedl ⁷⁵	70% ethanol	— 91.0°/20°
"	70% ethanol	— 91.3°/20°
Woodman ⁷⁶	70% ethanol	Manitoba flour — 93.6°
"	70% ethanol	English flour — 93.8°
Halton ⁷⁷	0.5N NaOH	Durum — 99.0°
"	0.5N NaOH	Fife — 99.2° to —100.1°
Blish and Pinckney ⁷⁸	70% ethanol	Kanred —100°/20°
"	70% ethanol	Marquis — 97.4°/20°
"	70% ethanol	Polish — 93.6°/20°
"	anh. acetic acid	Kanred — 85.8°/20°
"	anh. acetic acid	Marquis — 85.5°/20°
"	anh. acetic acid	Polish — 79.5°/20°
"	0.1N NaOH	Kanred —107.7°/20°
"	0.1N NaOH	Marquis —106.5°/20°
"	0.1N NaOH	Polish —102.2°/20°
"	0.1N NaOH	Kanred —109.9°/20°
"	0.1N NaOH	Marquis —110.6°/20°
"	0.1N NaOH	Polish —104.6°/20°

⁷⁰ *Forhandl. Skand. Naturfors.*, p. 385 (1892).⁷¹ *J. Am. Chem. Soc.*, **25**, 842 (1903).⁷² *J. Am. Chem. Soc.*, **28**, 1482 (1906).⁷³ *Z. physiol. Chem.*, **31**, 165 (1901).⁷⁴ *Compt. rend.*, **145**, 253 (1907).⁷⁵ *Biochem. Z.*, **66**, 154 (1915).⁷⁶ *J. Agr. Sci.*, **12**, 231 (1922).⁷⁷ *J. Agr. Sci.*, **14**, 587 (1924).⁷⁸ *Cereal Chem.*, **1**, 309 (1924).

Table 8.—Continued

Investigator	Solvent	(α) _D
Dill and Alsberg ⁷⁹	70% ethanol	— 89.9°/20°
Dill and Alsberg ⁷⁹	60% ethanol	— 91.0°/20°
"	50% ethanol	— 90.3°/20°
"	70% ethanol	— 93.7°/40°
"	60% ethanol	— 95.8°/40°
"	50% ethanol	— 94.2°/40°
"	50% ethanol	— 96.9°/50°
"	30% <i>n</i> -propanol	— 97.7°/20°
"	40% <i>n</i> -propanol	— 98.4°/20°
"	50% <i>n</i> -propanol	— 98.6°/20°
"	60% <i>n</i> -propanol	— 98.1°/20°
"	30% urea	— 116.5°
Dingwall ⁸⁴	70% ethanol	— 92.3°
Troensegaard ⁸⁰	58% ethanol	— 92.8° to — 94°
Haugaard and Johnson ⁸¹	60% ethanol	— 94.3°
Wiles and Gortner ⁸¹	5% ethanol	— 84.0°
"	10% ethanol	— 95.0°
"	70% ethanol	— 95.9°
"	water	— 93.0°
"	0.5 <i>M</i> KI	— 89.5°
"	0.5 <i>M</i> KBr	— 75.5°
"	0.5 <i>M</i> NaCl	— 73.3°
"	0.5 <i>M</i> LiCl	— 94.6°
Csonka, Horn, Jones ⁸²	0.5 <i>M</i> NaOH	— 112.7°/38°
"	0.5 <i>M</i> NaOH (after 240 hrs)	— 51.4°/38°

in a 400-mm tube and multiply the corrected reading by 0.215 to give the equivalent of gliadin nitrogen.

Several investigators reported on the specific rotatory power of gliadin, however, and a consolidation of the more significant findings are reported in Table 8.

Specific rotation of gliadin solutions in 0.5*N* NaOH was found by Woodman ⁸³ to decrease from — 111° at the 2nd hour to — 67° at the end of the 240th hour. When alcohol was present the total change in specific rotation was about the same, although the rate of change was much faster during the first 24 hours. In 0.25*N* NaOH, with alcohol present the specific rotation did not change so fast, but it fell to — 82° after about 240 hours. No differences were observed in these particulars between the gliadins prepared from a strong Manitoba wheat flour and a weak English wheat flour.

Rotation dispersion of gliadin in 70 per cent ethanol at 21.1° C was observed by Dingwall ⁸⁴ using monochromatic light at four different wave lengths, with the results shown in Table 9. Assuming that the earlier investigators employed a light source which emitted most strongly in the D line, Dingwall's observations at 5890 Å are in good agreement with

⁷⁹ *J. Biol. Chem.*, **65**, 279 (1925).

⁸⁰ *Z. physiol. Chem.*, **199**, 129 (1931).

⁸¹ *Cereal Chem.*, **11**, 36 (1934).

⁸² *J. Biol. Chem.*, **89**, 267 (1930).

⁸³ *J. Agr. Sci.*, **12**, 281 (1922).

⁸⁴ "Studies of proteins of wheat flour in relation to flour strength." Thesis filed in the Library of the University of Minnesota (1924).

those of Kjeldahl, Osborne and Harris, Mathewson, Kossel and Kutscher and others. Moreover, the gliadin preparations isolated from three widely different flours of "strong" high protein, and "weak" low protein types did not differ materially in these properties. In other words, those portions of the molecules which are responsible for optical rotation appear to be identical in the instance of these gliadin preparations.

Table 9. Rotation Dispersion of Gliadins Prepared from Strong and Weak Flours, in 70% Alcohol at 21.1° C as Observed by Dingwall.⁸⁴

Wave length (Å)	"Strong" hard wheat No. 8410	Soft wheat flour No. 8496	"Weak" cracker flour No. 8497
6500	— 72.7°	— 72.5°	— 72.1°
5890	— 92.9°	— 91.6°	— 92.3°
5300	— 120.7°	— 118.9°	— 120.0°
4800	— 151.7°	— 151.3°	— 151.3°

These studies of rotatory dispersion of gliadin were expanded by Wiles and Gortner,⁸⁵ who gave particular attention to the effect of the method of preparation of the gliadin and the peptizing solution upon this physical property. Their three gliadin preparations were those of Sinclair and Gortner,⁵⁵ described elsewhere in this chapter. No differences were observed between the three preparations, and, moreover, it developed that there was a remarkable agreement between the three series of data accumulated independently by Dingwall, Haugaard and Johnson, and Wiles and Gortner respectively. The actual specific rotatory power of these gliadins was determined, as might be expected, by the characteristics of the peptizing medium as shown by the data for the D line ($\lambda = 589.3 \text{ m}\mu$; $\nu = 5.089 \times 10^4$) observations recorded in Table 8. Wiles and Gortner emphasized the difficulties encountered in the use of the saline solutions as peptizing agents, due to the low concentration of the disperse on the one hand, and the turbidity of many preparations in the second instance.

Specific rotation equivalent, as defined by S. P. L. Sorensen, namely the angle through which the plane of polarization is turned by a 10-cm layer of a solution containing 1 milligram equivalent of protein nitrogen per cc, was determined for gliadin by Haugaard and Johnson.⁸¹ The observed values were in the range of 7.7° to 8.2° for gliadin when the monochromatic light source was in the sodium D line.

No differences in the absorption spectra of gliadin preparations made from 8 varieties of wheat and 3 genetic species was observed by Kondo and Yamada.⁸⁶ No effects of method of cultivation of the wheat, or differences between varieties, were disclosed.

⁸⁵ *Cereal Chem.*, **11**, 36 (1934).

⁸⁶ *J. Chem. Soc. Japan*, **57**, 1250 (1936); *Chem. Abst.*, **31**, 2514 (1937). Original not seen.

The addition of gliadin to acetic acid, potassium hydroxide solution, phenol, ethanol, and propanol increased the refractive index of the solvent in every case except with 75 per cent phenol, where a slight reduction appeared in the experiments of Robertson and Greaves.⁸⁷ Dingwall⁸⁴ determined the refractive index dispersion of the same three gliadin preparations employed in his studies of specific rotation dispersion (Table 9) with the results shown in Table 10. These are in fair agreement with the data of Robertson and Greaves.

Table 10. Refractive Index Dispersion of Gliadins in 70 Per Cent Ethanol, as Determined by the Pulfrich Refractometer at 23° C by Dingwall.⁸⁴

Preparation	5780Å	Wave length 5461Å	4358Å
Solvent only	1.36318	1.36420	1.37048
Flour No. 8410	1.37671	1.37784	1.38467
"a" value*	0.00165	0.00166	0.00173
Flour No. 8496	1.38081	1.38194	1.38889
"a" value*	0.00166	0.00167	0.00173
Flour No. 8497	1.37311	1.37422	1.38085
"a" value*	0.00165	0.00167	0.00173

* "a" value of Robertson is the alteration of the refractive index of the solvent effected by dissolving 1 gram of the substance in it.

Here again, as in the instance of Dingwall's rotatory dispersion determinations, no evidence is afforded of any substantial difference in the constitution of the gliadin molecules from different flour types insofar as these are revealed by the refractive index dispersion or the several "a" values. It must be recognized that, in the instance of both of these physical techniques, the error of observation is fairly large on the one hand, and presumably only a portion of the molecule may be involved in the second instance. This means that there may be differences which are not fully disclosed by these methods alone.

Molecular Weight of Gliadin

A great variety of methods have been employed in the effort to determine the molecular weight of proteins, including wheat proteins. Space does not permit a discussion here of the details of these methods, or the acceptability of the several measurements. It does seem appropriate to record certain observations recorded in the literature, however, and this has been done for gliadin. Table 11 provides a consolidation of several of the computations, together with a brief indication of the general type of method followed in each instance. Later researches may clarify the usefulness of these or other methods and serve to indicate which of the levels, *i.e.*, 15,000 or 125,000 or somewhere in between, is the most acceptable.

⁸⁷ *J. Biol. Chem.*, 9, 181 (1911).

Burk⁹² found that gliadin, unlike certain other proteins, is not dissociated into units of lower molecular weight by urea, as shown by his data in Table 11. In glycerol and urethane solutions there appeared to be an association of the gliadin into aggregates presumed to be formed from coagulated gliadin molecules. Burk concluded that gliadin is an inhomogeneous protein.

Table 11. Estimates of the Molecular Weight of Gliadin.

Investigator	Type of method	Mol. wt.
Osborne ⁸⁴	Assumption of 5 atoms of S	min. 15,568
Cohn, Hendry and Prentiss ⁸⁸	Calculated from amino acid units	min. 20,700
Haugaard, Johnson ⁹¹	Precipitation temperature	40,400
Lamm, Polson ⁸⁹	Diffusion constant	$M_r = 27,500$
Krejci, Svedberg ⁹⁰	Sedimentation equilibrium	34,500
Arrhenius ⁹¹	Dipole moment (relaxation time)	26,800
Burk ⁹²	Osmotic pressure, ethanol 50-70%	40,900
	" " 6.66 <i>M</i> urea	44,200
	" " 75% glycerol	67,000
	" " 15% urethane	75,300
McCalla, Gralen ⁹³	Sedimentation—diffusion and equilibrium.	(Lowest mol.) wt. fraction=43,000

Krejci and Svedberg⁹⁰ encountered at pH 2.23 and above, and at 20° C or lower what appeared to be mixtures of whole (mol. wt. 34,500) and half molecules (mol. wt. 17,250) of gliadin. At higher temperatures and higher acidities dissociation into half molecules was complete. When Haugaard and Johnson's gliadin fractions were examined, the least soluble fraction was found to contain a high concentration of heavy molecules, while the most soluble fraction was comprised almost wholly of the constituent of lowest molecular weight.

For detailed information concerning the methods employed by Svedberg and associates the reader is referred to "The Ultracentrifuge" by Svedberg and Pedersen.⁹⁴

The determination of the molecular weight of gliadin from its composition is discussed by Cohn, Hendry and Prentiss.⁸⁸ A tabulation of the estimated minimal molecular weight, based upon a stipulated number of molecules of several amino acids, or of a number of atoms of sulfur is recorded in Table 12. From these calculations they conclude that the minimum molecular weight lies in between 20,000 and 21,000, most probably in the neighborhood of 20,700. Values in the same general range were also computed based upon the base-binding capacity as measured

⁸⁸ *J. Biol. Chem.*, **63**, 721 (1925).

⁸⁹ *Biochem. J.*, **30**, 528 (1936).

⁹⁰ *J. Am. Chem. Soc.*, **57**, 940 (1935).

⁹¹ *J. Chem. Phys.*, **5**, 63 (1937).

⁹² *J. Biol. Chem.*, **124**, 49 (1938).

⁹³ *Nature*, **146**, 60 (1940).

⁹⁴ "The Ultracentrifuge," Oxford, The Clarendon Press, 1940. Note particularly pp. 386-87.

by Greenburg and Schmidt.⁹⁵ In a later communication Cohn⁹⁶ accepts the same minimal value but proposes a probable molecular weight of 125,000.

Table 12. Minimal Molecular Weight of Gliadin as Computed and Reported by Cohn, Hendry and Prentiss.⁹⁸

Content of	Amount of constituent present (%)	Wt. containing 1 atom or molecule (g)	Assumed number of atoms or molecules	Minimal molecular weight
Tryptophane	1.14	17,904	1	17,904
"	1.0	20,410	1	20,410
Lysine	0.69	21,173	1	21,173
Cystine	2.32	10,353	2	20,706
β -hydroxyglutamic acid	2.4	6,796	3	20,388
Sulfide sulfur	0.619	5,181	4	20,724
Histidine	3.35	4,630	4	18,520
Arginine	3.14	5,544	4	22,176
Tyrosine	3.5	5,174	4	20,696
Sulfur	1.027	3,123	7	21,861

Bull⁹⁷ pointed out the uncertain density of the water of hydration in estimating the "molecular weight" of proteins with the ultracentrifuge, although he conceded that this instrument is the most important tool yet devised for the physical study of proteins. Also he emphasized the impossibility of estimating actual experimental errors involved in such determinations. Where it involved a choice of different values, Bull indicated a preference for the rate sedimentation method.

In addition to the matter of mass, the significance of asymmetries of proteins was discussed by Bull. Using Neurath's technique he recorded the asymmetry of gliadin (mol. wt. 27,500) as 11.0 (anhydrous) and 7.3 (hydrated). Hydration reduced the asymmetry in all cases that were computed. Other characteristics of proteins, including the evidence for various types of patterns, are discussed in useful fashion by Bull.

Products of Hydrolysis of Wheat Prolamins

Three general types of methods have been employed in determining the products of hydrolysis of wheat proteins, (1) the Fischer ester method, (2) the Hausmann number, (3) Van Slyke's method. All of these, and several special methods devised for specific amino acids or groups of amino acids, have been applied to the study of flour proteins. Recently Brazier amplified and extended a method devised in Schryver's laboratory which takes advantage of the differences in solubility of metallic salts of the amino acids. In this monograph it is not possible to relate all of the details of these involved methods. None of them is as accurate and

⁹⁵ *Proc. Soc. Exp. Biol. Med.*, 21, 281 (1923-4).

⁹⁶ *J. Biol. Chem.*, 63, Supplement, xv-xvi (1925).

⁹⁷ "Advances in Enzymology. Vol. I. Protein Structure," pp. 1-42, 1941.

quantitative as is to be desired; moreover, it is evident that the conditions of hydrolysis, including the nature of the hydrolyzing agent, time of hydrolysis and possibly other factors have a bearing upon the chemical composition of the resulting hydrolyzate. Accordingly, the findings are

Table 13. Products of Hydrolysis of Prolamins of Wheat.

	Osborne ⁹⁴	Abderhalden and Samuely ⁹⁸	Kossel and Kutscher ⁹⁹ ; Kutscher ¹⁰⁰	Osborne and Guest ¹⁰¹
Glycine	0.00	0.68		0.00
Alanine	2.00	2.66		1.95
Valine	0.21	0.33		3.34
Leucine	5.61	6.0		6.62
Proline	7.06	2.4		13.22
Phenylalanine	2.35	2.6		1.80
Aspartic acid	0.58	1.24	0	0.14
Glutamic acid	37.33	27.6 (31.5)	18.54	43.66
Tyrosine	1.20	2.37	2.09	
Lysine	0.00	0.0	0.0	
Histidine	0.61	1.7	1.20	
Arginine	3.16	3.4	2.75	
Tryptophane	+	1.0±		
Serine	0.13	0.12		
Cystine	0.45			
Ammonia	5.11		4.1	5.22

not always in good agreement, and the complete and accurate analysis of these proteins is still to be reported. In the meantime we must make use of the data available, and certain of these have been consolidated, for convenience, in Tables 13 and 14.

The four gliadin samples studied by Cross and Swain,¹⁰⁵ to which

Table 14. Fractionation of the Products of Hydrolysis of Wheat Prolamins After the Method of Van Slyke.

	Van Slyke ¹⁰²	Osborne <i>et al.</i> ¹⁰³	Blish ¹⁰⁴	Cross and Swain ¹⁰⁵	Larmour and Sallans ¹⁰⁶
Ammonia N	25.52	24.61	26.13	26.38	25.1-25.8
Humin N	0.86	0.58	0.50		0.8- 1.0
Histidine N	5.20	3.39	6.77	7.38	3.7- 5.8
Cystine N	1.25	0.80	0.37	0.67	0.5- 1.0
Arginine N	5.71	5.45	4.55	4.64	5.2- 6.2
Lysine N	0.75	1.33	0.65	0.25	0.6- 1.2
Filtrate N					
mono-amino	51.98	51.95	53.63	52.97	52.3-54.4
non-amino	8.50	10.70	7.44	7.62	7.2- 9.9

⁹⁸ *Z. physiol. chem.*, **44**, 276 (1905).

⁹⁹ *Z. physiol. chem.*, **31**, 165 (1901).

¹⁰⁰ *Z. physiol. chem.*, **38**, 111 (1903).

¹⁰¹ *J. Biol. Chem.*, **9**, 425 (1911).

¹⁰² *J. Biol. Chem.*, **10**, 15 (1911).

¹⁰³ *J. Biol. Chem.*, **22**, 259 (1916).

¹⁰⁴ *J. Ind. Eng. Chem.*, **8**, 138 (1916).

¹⁰⁵ *J. Ind. Eng. Chem.*, **16**, 49 (1924).

¹⁰⁶ *Can. J. Research*, **6**, 38 (1932).

Table 14.—*Continued*

	Hoffman and Gortner ¹⁰⁷	Hoffman and Gortner ¹⁰⁸	Haugaard and Johnson ⁹¹	Jones and Gersdorff ¹⁰⁰ (bran)	Cook ¹¹⁰
Ammonia N	24.61	25.90	25.03– 26.27	23.00	25.4 –26.6
Humin N	0.87	0.66	0.38–0.64	1.18	0.001– 0.040
acid insol.			0.03–0.36		
acid sol.			0.28–0.46		
Histidine N	5.41	8.22	6.33–7.14	1.45	
Cystine N	1.68	0.44		1.23	
Arginine N	6.38	5.29	6.46–6.93	9.12	5.19 – 5.81
Lysine N	0.57	0.50		3.02	
Filtrate N					
mono-amino	53.49	52.23	53.34– 53.95	56.13	53.3 –56.1
non-amino	6.14	5.95		5.09	7.5 – 9.5

reference is made in Table 14, contained from 17.21 to 17.91 per cent of nitrogen. In addition to the conventional Van Slyke fractionation of the products of acid hydrolysis of these gliadins, the tyrosine and tryptophane contents were determined by the Folin and Looney method after hydrolysis with barium hydroxide solution. The tyrosine values are suspected to be a little high; they ranged from 5.04 to 5.10 per cent. The tryptophane ranged from 1.03 to 1.19 per cent. Thus the four preparations exhibited little variability in these chemical characteristics.

A slightly larger amount of tryptophane was found in gliadin by Folin and Looney¹¹¹ than by Abderhalden and Samuely,⁹⁸ namely 1.14 per cent, which was in about the same range as the highest values found by Cross and Swain.¹⁰⁵ Folin and Looney also reported a higher percentage of cystine by their colorimetric procedure than had hitherto appeared in the literature, 2.32 per cent. Cohn, Hendry and Prentiss⁸⁸ comment that if these estimates of cystine and tryptophane are both correct, the molecular weight of gliadin cannot be less than 72,000.

May and Rose,¹¹² using Ehrlich's colorimetric reagent, found 1.05 per cent of tryptophane in gliadin.

A novel procedure for the determination of tryptophane and tyrosine in proteins without the necessity of recourse to preliminary hydrolysis was proposed by Tillmans, Hirsch and Stoppel.¹¹³ The gluten proteins were found to contain rather large proportions of tyrosine. Gliadin extracted directly with cold 70 per cent ethanol contained 0.79 per cent tryptophane and 5.2 per cent tyrosine. Another preparation made by digestion with heat gave 1.18 per cent tryptophane and 6.5 per cent

¹⁰⁷ Colloid Symposium Monographs, 2, 209. Reinhold Publishing Corp., 1925.

¹⁰⁸ *Cereal Chem.*, 4, 221 (1927).

¹⁰⁹ *J. Biol. Chem.*, 64, 241 (1925).

¹¹⁰ *Can. J. Research*, 5, 389 (1931).

¹¹¹ *J. Biol. Chem.*, 51, 421 (1922).

¹¹² *J. Biol. Chem.*, 54, 213 (1922).

¹¹³ *Biochem. Z.*, 198, 379 (1928).

tyrosine. When this heated alcoholic extract was strongly cooled some protein collected, and on analysis this was found to contain 1.2 per cent tryptophane and 7.5 per cent tyrosine.

In an earlier paper from the same laboratory Tillmans and Alt¹¹⁴ reported 0.84 per cent of tryptophane in cold extracted gliadin, and 1.14 per cent in the gliadin extracted by refluxing with heat, 70 per cent alcohol being employed as the solvent.

The tryptophane and cystine contents of a number of proteins were determined by Jones, Gersdorff and Moeller,¹¹⁵ using the method described by May and Rose¹¹² for the former, and the Folin and Looney¹¹¹ method for the latter. Several samples of gliadin were analyzed, as well as a prolamin isolated from bran, and from several other species of the genus *Triticum*. The results of these determinations are recorded in Table 15.

Table 15. Percentages of Tryptophane and Cystine Found in Various Prolamins by Jones, Gersdorff and Moeller.¹¹⁵

	Tryptophane (%)	Cystine (%)
Gliadin from durum wheat furnished by R. A. Gortner	1.09	1.42
Gliadin furnished by R. A. Gortner	0.70	1.68
Gliadin furnished by G. E. Holm	1.09	1.56
Prolamin from wheat bran	1.37	2.29
" " einkorn	none	3.48
" " emmer	0.80	1.98
" " spelt	1.08	1.79

The cystine content of the bran prolamin is notably higher than that of the flour gliadin furnished by either Gortner or Holm, while the einkorn prolamin is still higher. On the basis of these analyses alone the prolamin prepared from spelt is quite similar to the gliadin furnished by Holm.

Methionine, in addition to cystine, was reported by Jones¹¹⁶ in gliadin and bran prolamin as follows:

	Cystine (%)	Methionine (%)
Gliadin	1.96	1.28
Bran prolamin	1.86	1.15

The cystine content of this gliadin preparation is somewhat higher than that of Jones' glutenin, which was 1.23 per cent. On the other hand, the glutenin contained more methionine, namely 1.56 per cent.

Jones and Wilson¹¹⁷ applied Foreman's¹¹⁸ method for precipitating the dicarboxylic acids of a protein hydrolyzate as their calcium salts with alcohol and recovered 53.6 per cent of the gliadin in this fraction. Of

¹¹⁴ *Ibid.*, 164, 135 (1925).

¹¹⁵ *J. Biol. Chem.*, 62, 183 (1924).

¹¹⁶ *Cereal Chem.*, 14, 771 (1937).

¹¹⁷ *Cereal Chem.*, 5, 473 (1928).

¹¹⁸ *Biochem. J.*, 8, 463 (1924).

this quantity 51.2 per cent was isolated as pure aspartic, glutamic, and hydroxyglutamic acids, leaving 2.4 per cent unaccounted for. They assume that this difference is due to an incomplete recovery of glutamic and hydroxyglutamic acids. The pure glutamic acid recovered represented 43 per cent of the gliadin, which is practically identical with that found by Osborne and Guest,¹⁰¹ *viz.*, 43.7 per cent. Aspartic acid equivalent to 0.5 per cent, and hydroxyglutamic equivalent to 7.7 per cent were recovered, although the latter figure is doubtless minimal, and less than the actual quantity present. The yields of glutamic and aspartic acids are of the same order as were reported by Jones and Moeller.¹¹⁹ As far as glutamic acid alone is concerned, gliadin hydrolyzates contain more of it than those of any other proteins studied by these investigators.

Errors arising in the determination of the dicarboxylic acids in protein hydrolyzates were discussed by Damodaran,¹²⁰ who proposed that Foreman's lime-alcohol method for precipitating them be introduced into the Van Slyke procedure. As applied by him to a sample of gliadin, the total dicarboxylic acid nitrogen was found to be equivalent to 28.0 per cent of the total nitrogen, in a preparation containing 17.3 per cent of total N, and yielding 25.7 of "amide" (ammonia) nitrogen.

Partition of the amino acid products of hydrolysis of gliadin was effected by Town¹²¹ and the dicarboxylic fraction was obtained by precipitation with baryta and alcohol. The yield of glutamic acid amounted to 40.4 per cent of the dry protein, or to 20.7 per cent of the nitrogen. In addition to the ordinary *l* (+) glutamic acid there was isolated a new variety, which was believed to be the racemic form, and was present in the ratio of 1:20 of the *l* (+) glutamic acid. A yield of pure aspartic acid was recovered from the dicarboxylate fraction equivalent to 0.43 per cent of the dry protein, or 0.26 per cent of the total nitrogen.

The coefficient of correlation of the total basic nitrogen, and the arginine nitrogen reported from 214 analyses of proteins by the Van Slyke method, including gliadin and glutenin, was computed by Larmour.¹²² The coefficient $r = + 0.794 \pm 0.017$. A similar computation of the correlation of total basic nitrogen and lysine nitrogen gave the value $r = + 0.548 \pm 0.032$. Larmour suggested that these computations afford indirect evidence of Kossel's hypothesis that arginine is the nucleus of the protein molecule.

Attention was called by Larmour and Sallans¹²³ to the fact that the sum of the ammonia nitrogen and the basic nitrogen in the hydrolyzate

¹¹⁹ *J. Biol. Chem.*, **79**, 429 (1928).

¹²⁰ *Biochem. J.*, **25**, 2123 (1931).

¹²¹ *Biochem. J.*, **35**, 417 (1941).

¹²² *Trans. Roy. Soc. Can. III*, **22**, 349 (1928).

¹²³ *Can. J. Research*, **6**, 38 (1932).

of gliadin (mean = 37.5%) is about the same as in the hydrolyzate of glutenin (mean = 36.7%).

The Hausmann nitrogen distribution method was applied by Sinclair and Gortner⁵⁵ to various gliadin preparations with the following results: Ammonia nitrogen, 25.3-25.8 per cent; humin nitrogen, 0.71-0.95 per cent; basic nitrogen, 10.5-11.0 per cent; non-basic nitrogen, 63.4-63.9 per cent.

An alcohol-soluble protein was isolated from wheat bran by Jones and Gersdorff,¹²⁴ the nitrogen of which represented 31 per cent of the total nitrogen of the bran. The average elementary analysis of five preparations on an ash- and moisture-free basis was: N 15.35, C 54.25, H 6.75, S 1.35, O 22.30 per cent. The percentage of nitrogen was significantly lower than that of the gliadin isolated from flour by Osborne (17.66), and of the numerous fractions recovered by Haugaard and Johnson, which ranged from 17.43 to 17.71 per cent in nitrogen content.

Teller and Teller¹²⁵ found only 10.3 per cent and 16.3 per cent of the total nitrogen in the prolamin fractions of spring wheat bran and soft winter wheat bran, respectively. The flours from the same wheats had 62 per cent and 67.5 per cent of their nitrogen in the prolamin fraction, when the latter was extracted with 60 per cent ethanol at 45° C and corrected by subtracting the non-protein nitrogen from the alcohol-soluble nitrogen. Thus it appears that there is appreciably less alcohol-soluble protein in the pericarp of the wheat kernel than in the endosperm.

In addition to the data recorded in Table 14, Haugaard and Johnson⁶¹ reported the following amino acids in their acid hydrolyzates of several gliadin fractions, in terms of the percentage of total nitrogen: tryptophane-N, 0.84-1.41; tyrosine-N, 0.86-1.32; proline-N, 11.01-12.96. Their calculation of glutamic acid, based upon the assumption that it is the only mono-amino, dicarboxylic acid present in the hydrolyzate (which is not exactly correct) gives a value of 52.77 per cent, which agrees well with the data of Jones and Wilson.¹¹⁷

Attention was called by Spörer and Kapfhammer¹²⁶ to the large variation in the proline content of gliadin reported in the literature, ranging from 2.04 per cent, as compared by Abderhalden and Samuely,⁹⁸ to 7.06 and 13.22 per cent, as reported by Osborne *et al.* Using an improved procedure, involving the recovery of the proline as a cadmium salt, Spörer and Kapfhammer found 9.86 per cent of that amino acid in their gliadin preparation.

In an extensive paper on the occurrence and recovery of hydroxy-glutamic acid from proteins, Dakin¹²⁷ states that 2.4 g of this acid was

¹²⁴ *J. Biol. Chem.*, 58, 117 (1923).

¹²⁵ *Cereal Chem.*, 9, 580 (1932).

¹²⁶ *Z. physiol. chem.*, 187, 84 (1930).

¹²⁷ *Biochem. J.*, 13, 398 (1919).

obtained from 100 g of gliadin, and, moreover, that undoubtedly the losses in recovery were considerable. The acid had all the properties of the β -hydroxyglutamic acid that was prepared from caseinogen.

Felix and Reindl¹²⁸ reported 0.77 per cent aspartic acid, 43.66 per cent glutamic acid, and 7.7 per cent oxyglutamic acid in the acid hydrolyzate of gliadin. In the basic group, 3.14 per cent of arginine, 3.35 per cent of histidine, and 0.69 per cent of lysine were found.

Hydrolysis of gliadin with water at 150°, and CO₂ under pressure of 10 and 20 atmospheres followed by fractionation into (I) soluble and (II) insoluble parts did not result in any difference in nitrogen distribution in I and II, according to Komatsu and Yukitomo.¹²⁹ Approximately half of the product of such manipulation was of a proteose-like nature. On increasing the time of digestion, more amino nitrogen and less mono- and diamino-nitrogen compounds were found. This was interpreted to indicate that the first cleavage products are diamino acids, from which simpler amino compounds can be produced by increasing the digestion period. In a more involved hydrolytic treatment, employing HCOOH, the hydrolyzate could be fractionated into a (III) soluble and (IV) an insoluble part, III having a higher N content, while IV had less amino acid and amide N, with no difference in diamino N.

Glutamine amide was recovered by Damodaran, Jaaback and Chibnall¹³⁰ from trypsin and yeast-dipeptidase digests of gliadin; they concluded that evidence was thus afforded that a dicarboxylic acid amide exists in the protein molecule. This received further confirmation at the hands of Syngé.¹³¹

Cyclic compounds were isolated by Troensegaard and Mygind¹³² from acetylated and hydrogenated gliadin which included (1) piperidine, (2) a base, C₁₁H₂₂N₂, isolated as the picrate, and (3) a base, C₁₄H₂₂N₂, isolated as the styphnate. Both bases contained only tertiary N, and combined with 2 mols of monobasic acid. These investigators thus find support for their theory that proteins, including gliadin, contain heterocyclic nuclei which yield amino acids on hydrolysis.

The issue as to whether or not gluten was comprised solely of gliadin and gluten was raised in definite form by Sandstedt and Blish.¹³³ They observed that a gluten dispersion in 55 per cent ethanol (by volume) and containing acetic acid to the equivalent of 0.05N, can be so manipulated as to distinguish three major types or groups of proteins: I. The "glu-

¹²⁸ *Z. physiol. chem.*, 205, 11 (1932).

¹²⁹ *J. Chem. Soc., Japan*, 52, 712 (1931); *Chem. Abs.*, 26, 4832. Original consulted but not read.

¹³⁰ *Biochem. J.*, 26, 1704 (1932).

¹³¹ *Biochem. J.*, 33, 671 (1939).

¹³² *Z. physiol. chem.*, 193, 171 (1930).

¹³³ *Cereal Chem.*, 10, 359 (1933).

tenin" group, which is but slightly soluble in either aqueous ethanol or aqueous acetic solutions (dilute), and is precipitated from its dispersion in a mixture of the two by the addition of neutral salts. When thus manipulated it appeared to constitute about one-fourth of the gluten. II. The "gliadin" group, soluble in neutral 50 to 70 per cent aqueous ethanol, as well as in dilute acetic acid. The neutral alcohol solutions are clear and filterable. This group constituted about one-half of the gluten. III. The "mesonin" fraction, appreciably, though not highly, soluble in neutral aqueous ethanol, readily dispersed by dilute acetic acid, or by ethanol containing dilute acetic acid and giving a clear, filterable sol. It appeared to constitute about one-fourth of the gluten. In view of its observed behavior on precipitating by cooling certain types of dispersions, several thermal manipulations have been employed in recovering it from gluten.

A preparation of the so-called "mesonin" was obtained by Stockelbach and Bailey¹³⁴ by dispersing wet crude gluten in 0.1N acetic acid, to which was added 1 g of K₂SO₄ per liter, and, after 24 hours, sufficient ethanol to bring the concentration to about 50 per cent by volume. A fraction, considered to be *glutenin*, settled out, and was recovered. The residual clear dispersion was then cooled to 12°-14° C, and a precipitate of *mesonin* appeared, which was also recovered. These two preparations were hydrolyzed with 20 per cent H₂SO₄ for 20 hours, and the hydrolyzate fractionated following much the same procedure that was described by Brazier,¹³⁵ but modified in certain details. The resulting data are recorded

Table 16. Nitrogen Distribution in Products of Sulfuric Acid Hydrolysis of the Mesonin and Glutenin Fractions from Wheat Flour Gluten, as Reported by Stockelbach and Bailey.¹³⁴

Fraction of hydrolyzate	Mesonin fraction (% total N)	Glutenin fraction (% total N)
Humins	1.26	4.47
Ammonia	17.69	13.42
Glutamic acid	15.17	6.33
Aspartic acid	2.32	3.37
Leucine	0.74	4.61
Phenylalanine	1.89	6.87
Lysine	—	1.82
Histidine	1.20	1.32
Arginine	6.95	9.74
Alanine	10.35	11.89
Proline	10.93	2.86
Serine	3.84	0.65
Hydroxyvaline	10.12	14.98
Valine	0.22	1.20
Bases	3.24	2.21
Cystine	—	2.34
Unidentified	1.72	4.30

¹³⁴ *Cereal Chem.*, 15, 801 (1938).

¹³⁵ *Biochem. J.*, 24, 1188 (1930).

in Table 16, and appear to indicate that the two fractions are quite different. Whether or not each fraction is comprised of a "pure" or homogeneous protein material, or are mixtures of several proteins is not apparent from this method of attack. The mesonin fraction resembles gliadin more closely than glutenin, particularly in view of its higher amide (ammonia) and dicarboxylic acid content. Assuming that the technique for the recovery of lysine is reasonably accurate, mesonin, like gliadin, contains little of that amino acid.

Two different fractionation techniques were applied by Harris and Bailey¹³⁶ to the recovery of three groups of proteins from gluten. Method A involved dispersing wet crude gluten in 0.1*N* acetic acid, and after standing in the laboratory for 16 ± hours, sufficient strong ethanol was added to bring the concentration to 50 per cent by volume. This was followed by the addition of 1 g of K₂SO₄ per liter. The *glutenin* was then allowed to precipitate at room temperature, recovered, and the quantity determined by Kjeldahling the precipitate. The remaining solution was cooled to 8° – 10° C for 24 + hours, and the precipitated *mesonin* was also recovered and Kjeldahled. The nitrogen content of the residual solution was determined, the factor N × 5.7 being applied in the instance of all three fractions. When this method was applied to 20 flour samples of widely varying characteristics both as to grade and strength, the range of fractionations, based upon percentage of total nitrogen in the original dispersion, was encountered that is recorded in Table 17.

Table 17. Range and Mean of the Glutenin, Mesonin, and Gliadin Fractions in *Vulgare* and *Non-vulgare* Wheat Flour Glutens, as Determined by Harris and Bailey,¹³⁶ Using Two Methods.

Method A.									
Wheat types	Average	Glutenin		Average	Mesonin		Average	Gliadin	
		Max.	Min.		Max.	Min.		Max.	Min.
<i>Vulgare</i>	5.5	13.7	3.2	35.9	43.2	19.5	58.6	77.3	47.2
<i>Non-vulgare</i>	4.6	8.5	2.5	26.4	42.0	6.8	69.0	89.2	54.6
Method B.									
<i>Vulgare</i>	34.7	42.6	29.1	13.4	16.3	9.2	51.9	55.1	45.4
<i>Non-vulgare</i>	30.7	33.7	25.6	11.6	17.3	7.5	57.7	62.3	50.4

Method B of Harris and Bailey differed from Method A in only one particular, namely, K₂SO₄ was added to the acetic acid dispersion before alcohol was added. This resulted in a much more copious precipitation of *glutenin*, as shown by the data in Table 17, and lends emphasis again to the indefiniteness of all these systems of protein fractionation. The *mesonin* fraction was reduced to almost the same degree as the *glutenin* was increased, there being the least difference in the *gliadin* recovery when

the two methods were compared. There was an indication of a higher content of gliadin in the non-*vulgare* than in the *vulgare* wheat glutes. The former included *Triticum compactum*, *T. spelta*, *T. durum*, *T. turgidum*, *T. polonicum*, *T. monococcum* and *T. emer.* Of these, *T. polonicum* raised at Pullman, Washington returned the highest percentage of gliadin from its gluten by Method A, but was not the highest by Method B.

Sugars in wheat gliadin were found by Kondo and Sarata¹³⁷ to consist of glucose, fructose and rhamnose. The ratio of these sugars was found to vary in varieties and genetic species, as well as in consequence of cultural conditions. The ratio was 1:1:3 for *T. monococcum*, 1:1:2 for *T. dicoccum* and *T. spelta*, and 2:2:1 for the Japanese variety of wheat.

A variety of methods have been employed by several researchers in the attempt to determine the isoelectric point of gliadin. Probably the lack of agreement in the findings is due partly to a failure to accept a common definition of the term, or rather, of the property, and partly to the inadequacy of certain assumptions respecting the interrelationship of various physicochemical properties of the complex molecules that are

Table 18. Isoelectric Point of Gliadin.

Investigators	Method	Isoelectric point, pH
Rona, Michaelis ¹³⁸	Flocculation and electrophoresis	9.23*
Eto ¹³⁹	Flocculation in buffer solns.	6.6
Tague ¹⁴⁰	Solubility	6.5
Kondo, Hayashi ¹⁴¹	Flocculation and precipitation	6.41-6.59
Hoffman, Gortner ¹⁰⁷	Potentiometric	5.76
" "	Acid and alkali binding I†	7.60
" "	" " " " II†	6.97
" "	" " " " III†	7.16
Csonka, Murphy, Jones ¹⁴¹	Flocculation in buffers	6.45
" " "	" " bran prolamin	6.20
Pearsall, Ewing ¹⁴²	Prec. from alcohol soln.	3.5-5.5 (5.2-?)
Wiazownicka ¹⁴³		6.2-6.4

* Calculated from $\text{CH} = 5.9 \times 10^{-10}$ in an NH_3 solution.

† Methods I-III involving the constants for the logarithmic curve $y = a + bx$. In I, $y = \log$ of the acid or alkali bound and $x = \text{the equilibrium pH}$; in II, $y = \log$ of the acid or alkali bound and $x = \log$ of acid or alkali added; in III, $y = \log$ of equivalents of acid or alkali added and $x = \text{pH}$ of the equilibrium solution. See the original paper for details. The authors comment that the values determined by Methods I, II and III are the isoelectric points as determined from the adsorption type of binding, or are the isoelectric points for the binding of acid above a hydrogen ion concentration of pH 2.5 and the binding of alkali above a hydroxyl ion concentration of pH 10.5. The "potentiometric" value is essentially the same as those indicated by the buffer curve of the dilute alkali or acid plus protein. Here the "isoelectric" point is not definite, i.e., a definite pH value, but covers a wide "isoelectric range."

¹³⁷ *J. Agr. Chem. Soc. Japan*, 16, 163 (1940); *Chem. Abs.*, 34, 4764. Original not seen.

¹³⁸ *Biochem. Z.*, 28, 193 (1910).

¹³⁹ *J. Biochem. (Japan)*, 3, 373 (1924).

¹⁴⁰ *J. Am. Chem. Soc.*, 47, 415 (1925).

¹⁴¹ *J. Am. Chem. Soc.*, 48, 763 (1926).

¹⁴² *Biochem. J.*, 18, 329 (1924).

¹⁴³ *Bull. Acad. Polonaise Sci. Lettres (B)*, 1933, 107.

involved. There are so many functional centers in the protein molecule where dissociation may occur, that a single, sharp point in terms of pH where all exhibit a minimum dissociation, or where the positive and negative charges on the zwitter-ion are exactly in balance, is too much to expect. The best we can hope to encounter is an *isoelectric range* through which the foregoing definitions apply, and even in measuring it, we must agree upon the criteria to be applied.

Certain data recorded in the literature are consolidated in Table 18, together with a notation of the general type of method employed. Since this is a large subject in itself, space does not permit a discussion of these methods, or their limitations. Broadly speaking, the minimum "solubility" or dispersion, and maximum flocculation as a function of pH appears to have been encountered in the range of pH $6.5 \pm$.

Kemp and Rideal¹⁴⁴ found the observed isoelectric point of gliadin to be dependent upon the electrolyte concentration. When the ionic strength ranged from 0.1000 to 0.0007 the isoelectric point pH ranged from 4.78 to 5.10. All the curves are smooth, which is assumed to be an indication that gliadin may be considered to be a monovalent ampholyte in respect to its acid and basic dissociation constants. They challenge the assumption that the solubility of gliadin is a minimum at the isoelectric point. Thus ultramicroscopic and turbidimetric observations of minimum solubility placed it between pH 6 and 7, which did not coincide with the isoelectric points indicated above.

A close relation was found by Greenburg and Schmidt¹⁴⁵ between the mols of NaOH bound by 1 gram of gliadin at pH 11 ($=30 \times 10^{-5}$) and the quantity computed from the sum of glutamic, β -hydroxyglutamic, and aspartic acids, and tyrosine minus the amide nitrogen ($=34 \times 10^{-5}$).

It was observed by Woodman¹⁴⁶ that 1 g of dry, ash-free gliadin required 1.95–1.99 cc of 0.1N NaOH to bring the dispersion to the isoelectric point of phenolphthalein, from which he computed the combining weight to be in the range of 5,000. From this the conclusion has been drawn that the gliadin molecule contains three, or a multiple of three, free carboxyl groups.

The acid and alkali binding capacity of gliadin and other prolamins was determined by Hoffman and Gortner,¹⁰⁷ using a method similar to that previously employed by Tague for amino acids, and by Loeb *et al.* for proteins. Acid or alkali sufficient to produce a solution of known normality was added to a solution of the protein, and the H-ion concen-

¹⁴⁴ *Proc. Roy. Soc. London*, A147, 1 (1934).

¹⁴⁵ *Proc. Soc. Exp. Biol. Med.*, 21, 281 (1924).

¹⁴⁶ *J. Agr. Sci.*, 12, 231 (1922).

tration of the resulting preparation was then determined by means of a hydrogen electrode. The difference between this value and the H-ion concentration of a protein-free solution otherwise identical was computed. In Table 19, when N is the original normality of the acid or alkali used, N' is the normality of the acid or alkali of the equilibrium solution in the presence of 1 per cent of the protein, $n = N - N'$, or the amount of acid or alkali bound by the protein.

Table 19. Acid and Alkali Bound by Gliadin (1 per cent) with Varying Normalities of HCl and NaOH, as Reported by Hoffman and Gortner.¹⁰⁷

With HCl		With NaOH	
N	n	N	n
0.003	0.002	0.005	0.003
0.009	0.003	0.020	0.005
0.018	0.006	0.040	0.012
0.030	0.009	0.060	0.008
0.060	0.015	0.100	0.017
0.090	0.023	0.140	0.036
0.120	0.038	0.200	0.060
0.180	0.051	0.300	0.086
0.240	0.042	0.400	0.113
0.300	0.058	0.500	0.126

The pH range in the HCl preparations is from 3.08 down to 0.666, and in the NaOH preparations from 11.25 up to 13.31.

The logarithms of the equivalents of acid (or alkali) bound by the proteins when plotted as ordinates (x) against the logarithms of the equivalents of acid or alkali added, or the final pH as abscissa (y) gave straight lines. The constants a and b for gliadin in the equation $y = a + bx$ were: with HCl, $a = 2.2612$ and $b = -0.7156$; with NaOH, $a = -10.3681$ and $b = 0.9302$.

Molar binding of gliadin, designated by Hirsch¹⁴⁷ under the symbol θ , was recorded in terms of the mols of NaOH or of HCl bound per mol of protein nitrogen at a definite pH stage. In other words, for a specific hydrogen ion concentration (H^+), there was also a definite, specific fraction of the acid or base split off. Holl¹⁴⁸ applied this concept to a gliadin preparation which he isolated from wheat flour by Alt's method and derived the value $\theta = 0.718$. Another preparation, made by Fleurent's method,¹⁴⁹ gave $\theta = 0.699$. Evidently this value for θ was based upon the equivalent quantity of normal NaOH bound by an alkali-hydrolyzed, and acidulated gliadin preparation in a stipulated pH range passing through pH=7 and computed in terms of millimols of protein nitrogen in the preparation as tested. The principal difference between this value, and the measurements made by other investigators is that it records acid- or

¹⁴⁷ *Biochem. Z.*, **147**, 433 (1924).

¹⁴⁸ Inaugural-Dissertation, Frankfurt University, 1926.

¹⁴⁹ *Ann. fals.*, **17**, 299 (1924).

alkali-binding capacity in terms of units of protein nitrogen, rather than in terms of weight of the entire protein substance.

Are Gliadin and Glutenin Distinct Proteins?

As early as 1895 O'Brien¹⁵⁰ observed that, in consequence of the varying proportions of gliadin that are recovered from flour by different methods, it is conceivable that one of the gluten proteins is derived from the other. He felt it is probable that the less soluble substance (called "zymom" by him) is derived from the more soluble, gliadin, "hence the alcohol-soluble substance (gliadin) is ultimately co-extensive with gluten." Also O'Brien suggests the existence of one protein mother-substance in flour which readily undergoes hydration, giving rise to gluten. This view is not altogether unlike that held by Weyl and Bischoff,¹⁵¹ who contended that flour contained a single protein substance, "vegetable myosin," which is converted into gluten by enzyme action. This matter of interconvertibility was supported by Kosutany,¹⁵² who proposed that gliadin may be considered to be the hydrate of glutenin, and glutenin in turn as the oxidized anhydride of gliadin.

The brilliant and extensive researches of the Osborne school, particularly during the last decade of the nineteenth century and the first decade of the twentieth, evidently tended to suppress further conjecture along these lines, and for a quarter century or more protein chemists and cereal technologists were more or less content to accept the idea that there were just two gluten proteins, possessed of distinctive properties and differing in chemical constitution to a degree that did not admit of interconvertibility. In fact, the latter idea tended to disappear.

Then researches began to appear in increasing volume which grew out of efforts to refine the quantitative methods for determining the several flour proteins. Attention was called to the large variations in the quantity of protein extracted by various concentrations of ethanol and other solvents in the instance of gliadin, and the uncertainties of interpretation of the findings based upon the attempts to remove non-gluten proteins from flour by extraction with neutral salt solutions. Certain of these and related investigations are detailed in the paragraphs which follow, and in the earlier discussion of the gliadin determination as such.

Kuhlmann¹⁵³ (also spelled Kul'man elsewhere) considers that gluten represents a complex of proteins forming micelles of various lengths, of which the longest and most stable are those of glutenin. Gliadin was more effectively peptized by 40-50 per cent ethanol than by more dilute or more

¹⁵⁰ *Ann. Bot.*, 9, 543 (1895).

¹⁵¹ *Ber.*, 13, 367 (1880).

¹⁵² "Der Ungarische Weizen und das ungarische Mehl," Budapest, 1907.

¹⁵³ *Nature*, 140, 119 (1937).

concentrated solutions in water. In another experiment the same gluten was treated progressively with a series of ethanol solutions of increasing concentration, and again the maximum peptization was effected with about 40 per cent ethanol.

On the other hand, when the proteins extracted by 40 per cent ethanol (I), and 70 per cent ethanol (II) were compared, it appeared that they differed with respect to their behavior toward water, *i.e.*, in swelling and hydration. Thus preparation I imbibed 1.63 g water per g dry weight, while preparation II imbibed 1.83 g in the instance of the protein extracted from the wheat variety *Lutescens* 0329. Preparation I from *Ferrugineum* wheat imbibed 0.91 g, while II imbibed 1.08 g. Kuhlmann concluded that gliadin is not a chemical individual, but is an adsorption complex of at least two fractions, of which α -gliadin constitutes the main mass of Osborne's gliadin, whereas β -gliadin approaches the properties of glutenin in swelling, peptization, and micelle length.

Kuhl¹⁵⁴ decided that the protein bodies extracted from flour by alcohol do not constitute a chemically pure protein substance. These proteins evidence a strong adsorbability. The relations between wheat gliadin and rye gliadin might be characterized as the equivalent of isomerism.

McCalla and Rose¹⁵⁵ observed that when wet crude gluten was treated with sodium salicylate solutions of varying concentration, progressively increasing proportions of protein were dispersed as the concentration of sodium salicylate in the solvent was increased from 1 to 8 per cent. At the latter level practically all the gluten proteins were dispersed, and the same was true of 10 per cent sodium salicylate solution. When MgSO_4 was added to a gluten dispersion in 10 per cent sodium salicylate solution, the percentage of protein present that was precipitated increased with the concentration of MgSO_4 , and practically all the protein was precipitated at the equivalent of 0.5 saturation with MgSO_4 . When the precipitated protein preparations thus recovered were hydrolyzed with 20 per cent HCl, it was found that the amide nitrogen (NH_3) increased with the percentage of protein precipitated (*i.e.*, with the concentration of added MgSO_4) until 80 per cent of the protein was precipitated, and then the percentage of amide nitrogen decreased sharply. Conversely, the arginine nitrogen in the hydrolyzates of the same preparations tended to decrease and then increase. This discontinuity is taken to indicate that the most soluble protein differs from the remainder. From this study these investigators are uncertain as to whether or not this most soluble fraction, representing 20-30 per cent of the total protein in flour, is one distinct protein or a mixture of several, or a protein complex. However, they do

¹⁵⁴ *Chem. Z.*, 57, 333 (1933).

¹⁵⁵ *Can. J. Research*, 12, 346 (1935).

find a basis for challenging the older assumption that gluten is composed of only two proteins, gliadin and glutenin. Thus the curves expressing the percentage of gluten peptized by a series of sodium salicylate solutions of increasing concentration is smooth and regular, whereas a break in the region of 50 per cent peptization might be expected if two distinctly different proteins were present. Likewise the regularity of increase in amide nitrogen and decrease in arginine nitrogen (up to 80 per cent precipitation with MgSO_4) tends to support the assumption that the major part of gluten consists of a single protein complex which may be separated into a great many fractions which differ progressively and systematically in both physical and chemical properties.

This study was extended by Spencer and McCalla,¹⁵⁶ who not only confirmed the earlier observation of a progressively increasing dispersion of gluten with increasing concentration of sodium salicylate in the dispersion medium, but reversed the process and effected progressive precipitation by dilution of a dispersion in 8 per cent sodium salicylate solution. Except for the most soluble 15 per cent, gluten protein appeared to consist of a complex that could be progressively fractionated.

Employing ultracentrifugal methods, Krejci and Svedberg⁹⁰ found that gliadin as extracted by 64 per cent alcohol is heterogeneous as to molecular weight. In general, the least-soluble fraction contained the highest concentration of heavy molecules. Lamm and Polson's¹⁵⁷ refractometric studies indicated that all but the most soluble gliadin fraction was non-uniform.

McCalla and Gralen⁹³ demonstrated that only part of the protein of gluten that is dispersed by sodium salicylate is in true molecular solution, since considerable proportions are rapidly sedimented out at a relatively low speed in the ultracentrifuge. Thus about 35 per cent of the gluten protein was dispersed in 2 per cent sodium salicylate, but less than half of this (14 per cent) was in molecular solution. The percentage of protein not precipitated at 300,000 times gravity increased progressively with the concentration of sodium salicylate in the dispersion medium, however, passing through a maximum at 12 per cent of the salt.

Diffusion constants were also determined with four fractions that were fractionated on the basis of solubility. The D_{20} of these were 2.14, 3.13, 4.12 and 4.83 (units of 10^{-7} in each instance), being lowest with the least soluble. The diffusion curves showed that each fraction was itself highly inhomogeneous and, accordingly, each value given represents a mean. The mean molecular weight of the most soluble fraction was 44,000 as determined by both the sedimentation diffusion method and the

¹⁵⁶ *Can. J. Research*, 16, 483 (1938).

¹⁵⁷ *Biochem. J.*, 30, 528 (1936).

equilibrium method. The weights of the other fractions were much higher and increased progressively with decreasing solubility.

These investigators contend that the shape of the molecules in the several fractions must be very different, and of many lengths. They find support for McCalla's earlier hypothesis that gluten proteins involve a system made up of components varying regularly and systematically in chemical and physical properties.

The experience of Haugaard and Johnson⁶¹ in the thermal fractionation casts further doubt on the homogeneity of "gliadin." Also the experience of Sandstedt and Blish,¹³² confirmed by the work of Stockelbach and Bailey,¹³³ indicated that a protein fraction intermediate in properties between gliadin and glutenin, although more nearly resembling the former than the latter in chemical constitution, could be recovered on the partition of gluten. The details of these last three studies are recorded in an earlier portion of the protein section of this monograph. The preparation of two glutenins, α -, and β -glutenin, from gluten by Csonka and Jones¹⁵⁸ is not without significance. These two glutenins, described in a later chapter, were decidedly different in chemical constitution, and yet could be expected to be present in the conventional glutenin preparation.

Using the moving-boundary electrophoresis technique of Tiselius, as applied to solutions of gliadin prepared by the method of Blish and Sandstedt,⁵⁰ it was concluded by Putnam, Briggs and Gortner¹⁵⁹ that the gliadin preparations were electrophoretically heterogeneous or heterophoretic. Complex formations occurred between the components which made it impossible to determine the number of components present, but there were at least two, and, in all probability, a much larger number in the gliadin proteih.

Convincing evidence against the existence of two definite protein species in gluten was found by McCalla and Gralen¹⁶⁰ in their ultracentrifuge and diffusion studies on gluten. Support was found for the earlier view that experimental evidence is incompatible with the idea of the existence of classical gliadin and glutenin. They could not state without qualification, however, that no major protein species exists in gluten, because it was impossible to study the molecularly dispersed protein of the less soluble (in sodium salicylate solutions) fractions free from aggregates. There appeared to be indications that the molecules were progressively longer with decreasing solubility. Variability in the physical properties of the various fractions could not be explained by assuming that

¹⁵⁸ *J. Biol. Chem.*, **73**, 321 (1927).

¹⁵⁹ Paper entitled "The electrophoresis of gliadin solutions" presented before the 1942 convention of the American Association of Cereal Chemists, Chicago, Illinois.

¹⁶⁰ *Can. J. Res.*, (Sec. C.), **20**, 130 (1942).

gluten contains three or four protein species appearing in varying proportions in the different fractions. Rather, the best hypothesis is that the gluten protein is a protein system which varies progressively and regularly in both chemical and physical properties.

As is inevitable in dealing with substances of the complexity and molecular size of these proteins, it is not possible to dispose of the issue here raised with entire definiteness at this time. That various gluten protein preparations are not "pure" in a strict chemical sense seems obvious. Whether these preparations are merely mixtures of a limited number of chemical entities, or are made up of a graduated and well-nigh infinite number of components, extending regularly in their chemical and physical properties from one extreme to the other, is not fully apparent. Then there is the third suggestion, namely that they are fairly readily interconvertible, or even that they arise from a common mother substance through enzyme action or other manipulation. This does not seem tenable, however, in view of the relatively large differences in chemical constitution disclosed by the hydrolysis of various protein preparations. It seems evident that further work must be done, and new or improved methods of greater precision must be applied before a decision, devoid of conjecture, can be reached in these matters.

Chapter 3

Glutelins of Wheat

When flour is treated with dilute saline solutions, followed by extraction with alcohol (60-70 per cent) there remains in the residue a protein or proteins that can be recovered by treatment with dilute alkali solutions, and then precipitating the dispersed protein by neutralizing with acid. Protein recovered by this or some related process, or which represents the residue after extraction of gluten with alcohol has been variously known as zymom, plant fibrin, gluten casein and otherwise. S. W. Johnson proposed the term *glutenin* to describe it, a term which was adopted by Osborne, and used subsequently by numerous workers. Still later the generic term *glutelin* was included in the list of simple proteins defined by the American Committee on Protein Nomenclature essentially as simple proteins, insoluble in all neutral solvents, but readily soluble in dilute acids and alkalies. Such proteins are encountered most prominently in the endosperm of the caryopsis or fruit of the grasses, and those which have been studied most extensively are oryzenin from rice, and glutenin of wheat.

In the literature of the proteins published around the turn of the century the term *glutenin* was commonly applied to the protein prepared or recovered from wheat by the type of manipulation just described. Later there appeared a tendency to use the generic term *glutelin* to an increasing degree. Conceivably this resulted, in part, from the growing uncertainty as to the chemical purity of the protein preparations thus obtained, and a disposition to use a generic term that had been defined only by a description of a method of preparation, rather than a specific name which suggests a pure substance. However that may be, the present author has included both terms in the discussion which follows, and with the intent of employing the term used by the investigators whose work is being reviewed in each instance. Also in the chapter heading the name *glutelins* is used in the plural because of the present uncertainty as to whether or not the protein preparations here discussed are comprised of one or several substances.

Glutenin of wheat flour was not uncommonly referred to as the "insoluble" protein, and in much of the earlier work the quantity present in flour was estimated either by subtracting the sum of the alcohol-soluble and

salt solution-soluble proteins from the total protein, or by Kjeldahling the residue from extraction with such solvents. The latter procedure was described by the early referee of the Association of Official Agricultural Chemists, Chamberlain,¹ who did not refer to this fraction as glutenin, but described it merely as an insoluble residue.

Ladd,² as A.O.A.C. associate referee, described the former procedure, while Harcourt³ two years later omitted reference to glutenin altogether, which suggests that no serious consideration was then being given to the direct determination of the glutelins.

Other investigators had applied such estimates of the glutelins of flour as were attempted to the residues remaining after the gliadin had been extracted from crude gluten by alcohol or some other solvent. Manget⁴ treated crude gluten with alcoholic potash solution from which the glutenin was precipitated by treatment with an excess of carbon dioxide. This procedure was based essentially upon the method of Fleurent.⁵ Neither of these chemists recovered the glutenin as such, however, but estimated it from the difference between the percentage of total gluten and the percentage of gliadin in the non-precipitated fraction. Such procedures applied to crude gluten came to be regarded as doubtful quantitative methods, however, because of (a) the possible losses of proteins sustained in the initial recovery of the crude gluten, and (b) the presence in the latter of proteins and other substances in addition to gliadin and glutenin.

Glutenin was determined by Snyder⁶ in the residue resulting from the extraction of gliadin with 70 per cent alcohol. This residue was washed with more alcohol until protein-free, and then extracted with 5 per cent NaCl solution to remove the non-gluten proteins. Glutenin was then removed from the residue of the second extraction by dispersion in 0.2 per cent KOH, filtering the resulting solution, and Kjeldahling an aliquot. Snyder comments that, for all practical purposes, the glutenin can be estimated by difference.

The latest edition of Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (5th ed., 1940) recognizes two tentative methods for the determination of glutenin in flour. Method I merely involves the deduction of the sum of the K_2SO_4 -soluble nitrogen and the 70 per cent ethanol-soluble nitrogen from the total nitrogen, and multiplying the difference by 5.7 to give the glutenin in per cent.

Method II includes the extraction for one hour of 8 g of flour with a solution of 0.2g Ba(OH)₂ dissolved in 50 ml of carbon dioxide-free water,

¹ U. S. Dept. Agr., Bur. Chem. Bull. 81, p. 118 (1904).

² U. S. Dept. Agr., Bur. Chem. Bull. 122, p. 58 (1908).

³ U. S. Dept. Agr., Bur. Chem. Bull. 137, p. 149 (1911).

⁴ Rev. Internat. Patisf., 16, 91 (1902).

⁵ Compt. rend., 123, 755 (1896).

⁶ Minn. Agr. Exp. Sta. Bull. 63, p. 525 (1899).

addition of 96 per cent methanol (preferably synthetic) to make to a volume of 205 cc, immediate filtration through a cotton plug, and Kjeldahling of 50-cc aliquot of clear filtrate. Nitrogen in this filtrate is subtracted from the total nitrogen and the difference is regarded as glutenin nitrogen, which, multiplied by 5.7, is taken as the percentage of glutenin in the flour.

In addition to the barium hydroxide-methanol method of the A.O.A.C. for the determination of glutenin, the 1941 edition of *Cereal Laboratory Methods*, edited by a committee of the American Association of Cereal Chemists, includes (Chapt. III, Sect. 6) an alkali-methanol method as well. This provides for the dispersion of the proteins of 5 g flour in a mixture of 5 ml of 1.0N NaOH and 50 ml of water. After shaking at intervals for one hour, sufficient methanol is added to bring to a total volume of 205 cc. The starch is allowed to settle, the liquid decanted through a cotton plug, and 50 ml of the filtrate is withdrawn. Sufficient 0.2N HCl is then added to bring the pH to 6.4, as shown by the change of color in bromthymol blue to a light olive. The glutenin which precipitates is allowed to settle, recovered by centrifuging, and Kjeldahled. The nitrogen in the precipitate multiplied by 5.7 is recorded as the equivalent of glutenin.

The glutenin content of eleven flours was reported by Sharp and Gortner⁷ as ranging between 4.19 and 7.45 per cent. The glutenin was determined by first extracting the flour with 5 per cent K₂SO₄ solution, then extracting the residue with 70 per cent ethanol. The sum of the nitrogen in these two fractions was subtracted from the total nitrogen of the flour and the difference was assumed to represent the glutenin nitrogen, which, multiplied by 5.7, was recorded as glutenin. The total range in glutenin content as indicated above appears quite large; but this was chiefly in consequence of the substantial variation in the protein content of these flours. When the glutenin percentage as reported was divided by the total protein content in each instance, to give the percentage of the latter represented by the glutenin fraction, the range was from 41 to 45 per cent. This is a relatively small variation, particularly in view of the probable errors of the individual fractionations.

A refinement of the Sharp and Gortner method was proposed by Sharp and Herrington.⁸ Flour was extracted with 5 per cent K₂SO₄ solution and 70 per cent ethanol by weight. In each instance the extract was completely decanted from the residue and the bottle and residue were then weighed. From this weight was subtracted the weight of the bottle, plus the weight of the original charge of flour, and the difference was assumed

⁷ *Minn. Agr. Exp. Sta. Tech. Bull.* 19 (1923).

⁸ *Cereal Chem.*, 4, 249 (1927).

to indicate the quantity of solution still remaining in the residue. These were designated as X and Y respectively.

The flour residue was then washed with water into a Kjeldahl flask and subjected to a conventional nitrogen determination, and the quantity of nitrogen multiplied by 5.7. This quantity (Z) was then corrected by subtracting the protein assumed to be present in the extracts. If X and Y represented the computed volume of solutions remaining in the residues as indicated above; M , the percentage of 5 per cent K_2SO_4 -soluble protein; and N the percentage of alcohol-soluble protein, then:

$$\text{Glutenin} = \text{Protein in residue } (Z) - (0.01 MX + 0.0067 NY)$$

Difficulties attendant upon the recovery of unaltered glutenin from wheat gluten when alkali was used to disperse the glutenin were discussed by Blish and Sandstedt.⁹ They presented a new procedure for the preparation of glutenin which involved macerating wet crude gluten in a large volume of dilute acetic acid. The proteins present were completely dispersed, and, since this dispersion could not be filtered, it was directly diluted with methanol until a concentration of 65-70 per cent of the latter was reached. This solution was then passed through a supercentrifuge which removed much of the starch and other suspended matter. Normal NaOH solution was then added to neutralize the acid to a pH slightly below 7, when a heavy gelatinous protein precipitate was formed. The gliadin remained in the alcoholic solution and was decanted off at once. The protein precipitate could then be redispersed in dilute acetic acid, methanol added as before, and then reprecipitated by neutralizing with NaOH. In fact, this redispersion and reprecipitation could be repeated two or three times in all to effect a purification of the solution.

Preparations recovered after this fractionation procedure still contained traces of starch, but they had a nitrogen content as high as 17.4 per cent, and, accordingly, the dilution with carbohydrates must have been very small. On hydrolysis the amide (ammonia) nitrogen represented about 22 per cent of the total nitrogen, which is decidedly higher than most of the other preparations previously reported upon, and only about 4 per cent below the values commonly reported for highly purified gliadin preparations. Moreover, the arginine nitrogen in the hydrolyzate as determined by Plimmer and Rosedale's¹⁰ method was only about 9 per cent, which was lower than most of the earlier analyses.

Blish and Sandstedt¹¹ and Blish, Abbott and Platenius¹² instituted studies which led to a direct method for the preparation and quantitative

⁹ *J. Biol. Chem.*, **85**, 195 (1929).

¹⁰ *Biochem. J.*, **19**, 1020 (1925).

¹¹ *Cereal Chem.*, **2**, 57 (1925).

¹² *Cereal Chem.*, **4**, 129 (1927).

estimation of glutenin in flour. Two procedures emerged from these studies which were essentially as follows (see "Cereal Laboratory Methods," Amer. Assn. Cereal Chemists, 3rd ed., 1935):

(A) Flour was treated with 0.2*N* NaOH, and then sufficient methanol was added to bring to the equivalent of about 75 per cent by volume. An aliquot was then neutralized with 0.2*N* HCl against bromthymol blue indicator to a pH of $6.4 \pm$. The precipitated glutenin was recovered by centrifuging, Kjeldahled, and the nitrogen equivalent multiplied by the factor 5.7.

(B) Powdered Ba(OH)₂ (0.2 g) was added to the flour (8 g), followed by 50 cc of water. After a suspension formed, the volume was brought to 205 cc with methanol, immediately filtered, and an aliquot Kjeldahled. Since glutenin was presumed to be insoluble in the mixed barium hydroxide-methanol medium, the difference between the total nitrogen and the nitrogen of the filtrate was returned as glutenin nitrogen. Obviously this is not a direct method, as is the case with Method A above. This is essentially the method that was adopted as Tentative II by the Association of Official Agricultural Chemists, and is described in their "Book of Methods," (Fifth Edition, 1940). Tentative Method I involves the determination of glutenin by difference, by deducting the sum of the K₂SO₄-soluble nitrogen and the alcohol-soluble nitrogen from the total nitrogen and multiplying the difference by 5.7.

Grewe and Bailey¹³ estimated the glutenin content of a series of 17 wheat flours of widely varying composition, using two methods: the Sharp and Gortner method (I), and the direct method of Blish and Sandstedt (II),¹¹ involving dispersion of the proteins in dilute NaOH solution, addition of methanol, neutralization with HCl, and recovery of the precipitated glutenin by the use of the centrifuge. The two methods gave practically identical results with all but three samples, when the differences were +0.62, +0.58 and +0.97 per cent respectively, in favor of the Blish and Sandstedt method. Incidentally, the greatest difference appeared in the instance of a *durum* wheat flour, although whether or not the genetic species is significant is not apparent from these studies. It is also notable that the ratio of Glutenin I/total protein, and the ratio Glutenin I/Glutenin I+Gliadin of the individual samples varied only slightly about the means of the ratios, which were 0.37 and 0.46 respectively. In other words, the ratio of glutenin to total protein, and the ratio of glutenin to the sum of glutenin plus gliadin as thus determined, appeared to be fairly constant, particularly in view of the lack of great analytical precision in the determinations themselves.

¹³ *Cereal Chem.*, 4, 230 (1927).

Teller and Teller¹⁴ estimated the glutelin fractions of wheat, flour and bran by digesting the material with 0.2 per cent NaOH, determining the nitrogen content of the extract, then subtracting from the latter, the sum of the nitrogen of the albumin, globulin, prolamine, and non-protein fractions. This difference was recorded as glutelin nitrogen, and the data resulting from such an analysis of spring and winter wheats and the flour and bran milled from them are recorded in Table 20. Considering the errors of the method involved, it would appear that the "glutelin," as thus estimated by difference, is present in about the same proportion of the total protein in the pericarp and endosperm of wheat.

Table 20. Glutelin Nitrogen, as Percentage of Total Nitrogen, in Wheats, Flours and Brans, as Determined by Teller and Teller.¹⁴

Material	Total N (%)	Glutelin N as percentage of total N
Spring wheat	3.10	26.5
Spring wheat flour	2.92	26.7
Spring wheat bran	3.12	23.4
Soft winter wheat	2.28	26.8
Soft winter wheat flour	1.91	20.4
Soft winter wheat bran	2.53	26.2

Wheat kernels that had been fed upon by the granary weevil, so that only the aleurone and bran were left, were subjected to a fractionation of the proteins by Kretovitch.¹⁵ The material contained 3.54 per cent of nitrogen on the dry basis. Of this nitrogen, 13.28 per cent was in the protein of water extract (I). The residue, on extraction with 5 per cent K₂SO₄ solution, yielded 5.09 per cent of the original nitrogen to the extract (II). Then the residue was treated with 70 per cent alcohol, and 5.85 per cent of the nitrogen appeared in this extract (III). Finally, 0.2 per cent KOH solution was applied to the residue from (III); 27.10 per cent of the nitrogen was in that extract (IV), leaving 22.60 per cent in the final residue (V). This led Kretovitch to conclude that glutenin and albumin comprise most of the proteins contained in the tissues of this material, while little gliadin was present.

No clear-cut distinctions between the solubilities of gliadin and glutenin in 30 per cent urea solutions could be found by Cook and Rose.¹⁶ With crude gluten, 10 and 12 per cent sodium salicylate solutions appeared to effect more rapid and complete dispersion than higher concentrations. Some progressive denaturation of albumin was also evident in their studies in the presence of both urea and sodium salicylate solutions of fairly high concentration.

¹⁴ *Cereal Chem.*, **9**, 560 (1932).

¹⁵ *Biochem. J.*, **27**, 1687 (1933).

¹⁶ *Nature*, **134**, 380 (1934).

Preparation and Composition of Glutenin

The glutenin prepared by Osborne¹⁷ for the study of its products of hydrolysis was made from the residue of crude gluten after extracting with ethanol to remove the gliadin. This residue, after drying and grinding, was further extracted with absolute ethanol and ether. The resulting powder was then dispersed in 0.2 per cent KOH, filtered, and neutralized with dilute HCl. The precipitate was extracted with 70 per cent ethanol as long as any protein dissolved, and the extracted material was dehydrated with absolute ethanol, and dried over H₂SO₄. In certain preliminary studies reprecipitations were made, but the resulting glutenin preparations did not differ materially in elemental composition. The products of hydrolysis as determined by Osborne are recorded in Table 5 (Chapter 1).

"Gluten-casein" is that fraction of gluten that, according to the Ritt-hausen classification, is least soluble in dilute ethanol, but is soluble in dilute acids, and hence is approximately identical with "glutenin." Its products of hydrolysis were reported by Kutscher¹⁸ as follows: ammonia, 2.64 per cent, histidine 1.56 per cent, arginine 4.54 per cent, lysine 2.0 per cent, tyrosine 2.75 per cent, glutamic acid 9.0 per cent.

Glutenins prepared from four flour samples by Cross and Swain¹⁹ were found to contain from 16.23 to 16.81 per cent of total nitrogen. In addition to subjecting the products of acid hydrolysis of these glutenins to the conventional Van Slyke fractionation, with the results shown in Table 21, they also determined the percentage of tyrosine, which ranged from 5.34 to 5.92 per cent, and of tryptophane, which ranged from 1.55 to 1.61 per cent. These determinations were made by the Folin and Looney²² method, after hydrolysis of the glutenins for 48 hours with barium hydroxide solution; Cross and Swain comment that the tyrosine values are probably a little high because of the residual color of the reagent.

The tryptophane and cystine content of a glutenin preparation were determined by Jones, Gersdorff and Moeller,²⁰ using the method described by May and Rose²¹ for the former, and the Folin and Looney²² method for the latter, with the following results: tryptophane 1.72 per cent, cystine 1.56 per cent. The tryptophane content here reported is higher than that found for gliadin by the same investigators, while there was less difference in the cystine content of the two gluten proteins.

¹⁷ "The Proteins of the Wheat Kernel," Carnegie Institution of Washington (1907).

¹⁸ *Z. physiol. Chem.*, **38**, 111 (1903).

¹⁹ *Ind. Eng. Chem.*, **16**, 49 (1924).

²⁰ *J. Biol. Chem.*, **62**, 183 (1924).

²¹ *J. Biol. Chem.*, **54**, 213 (1922).

²² *J. Biol. Chem.*, **51**, 421 (1922).

Table 21. Products of Hydrolysis of Glutenin Fractionated by the Van Slyke Method.

Substance	Per cent total N		
	Blish ²³	Cross and Swain ¹⁹	Hoffman and Gortner ²⁴
Ammonia N	16.17-16.50	13.11-15.98	13.56
Humin, acid insol., N		0.52- 0.67	0.78
" sol., N		0.80- 1.07	0.66
total	1.66- 1.84	1.43- 1.74	1.44
Histidine	5.47- 7.59	6.20-11.42	4.72
Cystine	0.18	0.65- 0.72	0.67
Arginine	9.27- 9.69	8.17-12.94	11.96
Lysine	1.90- 2.61	4.72- 6.42	4.52
Filtrate N			
Amino	53.38-53.59	54.02-56.55	53.47
Non-amino	9.35- 9.52	2.64- 3.98	7.50
Total N in protein		16.23-16.81	17.20

In an extension of this discussion of glutenin, and other flour proteins, Blish²⁵ places quotation marks about the adjective "quantitative" as applied to the two methods proposed earlier by himself and his colleagues. He suggests that glutenin as prepared and described by Osborne is not a chemical individual and that we might do well to regard it as a type of substance. The existence of a third type of substance was suggested, and this probability received further emphasis by Sandstedt and Blish.²⁶

Larmour²⁷ prepared glutelins from several species of the genus *Triticum*, as well as other cereals. In the instance of common wheat, a modification of Osborne's method was followed. The crude glutenin from the separation of gliadin was dispersed in 0.2 per cent NaOH and precipitated by neutralizing with 0.2 per cent HCl. This was repeated and the coagulum was then washed with alcohol several times, and with ether, then dried and ground. On an ash- and moisture-free basis it contained 16.64 per cent of nitrogen.

In Table 22 are recorded the results of Larmour's study of the products of acid hydrolysis of the glutelins from several species, as determined by the Van Slyke method. A substantial difference in the amide (ammonia) nitrogen fraction was encountered, *T. vulgare* and *T. durum* being highest, and *T. spelta* lowest. The latter, on the other hand, yielded the most humin, but interpretations of such data are difficult, since it has been demonstrated that they are affected by the quantity of carbohydrates and other substances in the preparation undergoing hydrolysis. The lysine fraction is higher than is generally reported for gliadin, and the ammonia fraction is lower.

²³ *J. Ind. Eng. Chem.*, **8**, 138 (1916).

²⁴ *Cereal Chem.*, **4**, 221 (1927).

²⁵ *Cereal Chem.*, **7**, 421 (1930).

²⁶ *Cereal Chem.*, **10**, 359 (1933).

²⁷ *J. Agr. Res.*, **35**, 1091 (1927).

Table 22. Nitrogen Distribution of Glutelins Prepared from Several Species of the Genus *Triticum*, Determined by the Van Slyke Method by Larmour.²⁷

	<i>T. vulgare</i>	<i>T. durum</i>	<i>T. spelta</i>	<i>T. dicoccum</i>	<i>T. monococcum</i>
	% Nitrogen				
Ammonia N	14.78	13.25	8.06	11.06	10.78
Humin N	1.70	2.49	5.02	4.63	6.04
Nitrogen of bases					
Arginine	10.90	11.00	13.43	13.03	11.86
Cystine	0.40	0.88	0.41	0.75	1.04
Histidine	1.67	5.84	2.80	4.80	2.44
Lysine	5.83	3.48	7.80	6.02	4.82
Total	18.80	21.20	24.44	24.60	20.16
Nitrogen in filtrate					
Amino	58.15	54.47	56.35	54.41	56.66
Non-amino	5.39	9.01	5.72	4.93	6.60

Spörer and Kapfhammer²⁸ reported 5.98 per cent of proline in their glutenin preparation.

Dakin²⁹ obtained 1.8 g of hydroxyglutamic acid from 100 g of glutenin, which low yield may have been occasioned by losses in recovery. Moreover, the properties of the acid suggested that partial racemization had occurred incidental to the effect of alkali used in the preparation of the glutenin.

Glutamic acid constituted 25.7 per cent of the glutenin preparation of Jones and Moeller,³⁰ and 2.0 per cent was aspartic acid. They used improved methods developed by them for the separation and recovery of these dicarboxylic acids. These yields were higher, particularly in the instance of the aspartic acid, than had been obtained previously by the ester method.

After suggesting that acid or alkali, or a pretreatment with alcohol in the recovery of glutenin, may effect an alteration of both chemical and physical properties, Cook and Alsberg³¹ used a 30 per cent solution of urea to disperse wheat flour gluten. Starch was removed by a super-centrifuge. Glutenin could be precipitated from this solution by adding ethanol to a concentration of 70 per cent by volume, or methanol to about 60 per cent, but only a part of the precipitated protein could be redispersed in urea solution. Magnesium sulfate was then used as a precipitating agent, the equivalent of 0.17 of saturation being adequate. Dilution of the urea solution to a concentration of 10 per cent of urea was also effective in precipitating the glutenin. The salting-out procedure resulted in preparations having a nitrogen content of 16.4 and 17.0 per cent, while three preparations made by dilution contained 15.8, 16.3 and 17.3 per cent respectively. On hydrolysis, the nitrogen distribution was

²⁸ *Z. physiol. Chem.*, 187, 84 (1930).

²⁹ *Biochem. J.*, 13, 398 (1919).

³⁰ *J. Biol. Chem.*, 79, 429 (1928).

³¹ *Can. J. Research*, 5, 355 (1931).

as follows: amide (ammonia) N, 16.4-19.9 per cent; humin N, 0.3-1.3 per cent; arginine N, 9.6-11.1 per cent; amino N in filtrate, 54.7-60.7 per cent; non-amino N in filtrate, 0.5-7.4 per cent.

Dispersion of gluten in acid and alkali resulted in a loss of its original properties, in the experience of Rose and Cook.³² The use of neutral solvents effected less alteration. They observed that the viscosity of gluten in urea and sodium salicylate solutions was much higher than that of dispersions of the same concentration in acid or alkali. This indicated either a greater solvation or a lower degree of dispersion in the urea and sodium salicylate solutions, and the latter seemed more probable.

When heat treatments were applied to such gluten dispersions, Cook and Rose³³ observed changes due not alone to the action of heat, but also to the action of the solvent on the protein. The greatest changes effected by heat alone occurred at temperatures of 70° C or higher. This was true of flour alone, and also of gluten dispersed in neutral solvents. When heated in acid or alkali solutions the protein was altered to such an extent that it no longer exhibited the properties of native gluten.

Alkali solutions effected a more extensive hydrolysis of gluten than dilute acetic acid, in the studies of Cook and Rose,³⁴ as evidenced by the larger percentage of the total nitrogen not precipitated by tannic acid, and by trichloroacetic acid. The rates of hydrolysis as $K \times 10^6$ (unimolecular) were as follows:

0.1N NaOH at 30° C	$K \times 10^6 =$	1.7
NaOH at 80°		150.
0.1N CH ₃ COOH at 30°		0.55
CH ₃ COOH at 80°		1.2

The dilute NaOH did not carry the hydrolysis to completion, however, as 27 per cent of the original nitrogen was still in the form of protein after 9 hours at 80° C.

Chemical Constitution of Wheat Glutelins

Glutenin was prepared from wheat flour by Damodaran³⁵ by washing crude gluten with 73 per cent alcohol to remove the gliadin. The residue was dispersed in 0.2 per cent NaOH, and precipitated by dilute acetic acid. This was repeated, and the precipitated glutenin was then washed with dilute alcohol, and dehydrated with acetone. It contained 17.02 per cent nitrogen on an ash- and moisture-free basis.

³² *Can. J. Research*, **12**, 63 (1935).

³³ *Ibid.*, **12**, 238 (1935).

³⁴ *Can. J. Research*, **12**, 248 (1935).

³⁵ *Biochem. J.*, **25**, 190 (1931).

The glutenin preparation was then hydrolyzed with 25 per cent H_2SO_4 for 24 hours, diluted with 3 volumes of water and filtered to remove the humin. Ammonia was determined in an aliquot. The technique devised in Schryver's laboratory as reported by Brazier,³⁶ which involves the separation of certain of the amino acids on the basis of differences in the solubility of their copper salts, was then applied. This is the same general method that was used later by Stockelbach and Bailey.³⁷ While the method may be open to some criticisms, in the hands of Damodaran it gave higher yields of many of the mono-amino acids than had previously been reported. His data for glutenin are recorded in Table 23.

Table 23. Percentage of Total Nitrogen of Hydrolyzate of Glutenin in Certain Amino-acids, as Determined by Damodaran,³⁵ Using the Copper Salt Method of Brazier.³⁶

Humin N (%)	1.17	Arginine and histidine (%)	11.60
Ammonia N (%)	19.10	Glycine (%)	0.83
Tyrosine N (%)	1.91	Alanine (%)	5.68
Valine N (%)	0.72	Aspartic acid (%)	1.15
Proline N (%)	4.39	Leucine (%)	3.96
Glutamic acid N (%)	14.78	Phenylalanine (%)	1.37
Hydroxyglutamic acid N (%)	0.50		

After carefully investigating the accuracy of the methods involved, Folin and Looney²² reported 1.68 per cent of tryptophane in glutenin and 1.80 per cent of cystine. Their estimate of tryptophane is slightly lower than that made by May and Rose,²¹ which was 1.80 per cent.

Csonka and Jones³⁸ extracted crude gluten from wheat flour with alcohol (70 per cent by volume) in nine successive portions. The residue was then dispersed in 0.2 per cent NaOH, centrifuged and filtered, and saturated $(\text{NH}_4)_2\text{SO}_4$ solution was added to give an equivalent of 0.018 to 0.02 saturated solution. The α -glutelin which precipitated was recovered, redispersed and again precipitated, then washed with dilute alcohol, absolute alcohol, ether, and dried. A total of 20 g of dried α -glutelin was recovered from an original charge of 2,000 g of flour. It contained 17.14 per cent of nitrogen.

To the supernatant liquid left after precipitating the α -glutelin, additional saturated $(\text{NH}_4)_2\text{SO}_4$ solution was added to bring the concentration of the latter to the equivalent of 0.18 saturated. β -glutelin was then precipitated, which was recovered and purified in essentially the same manner as the α -glutelin. A total of 1.35 g containing 16.06 per cent of nitrogen was obtained, although a modified procedure later gave a larger yield of the same nitrogen content. Evidently the α -glutelin, as described

³⁶ *Biochem. J.*, **24**, 1188 (1930).

³⁷ *Cereal Chem.*, **15**, 801 (1938).

³⁸ *J. Biol. Chem.*, **73**, 321 (1927).

by this fractionation technique, constituted the bulk of the glutelins in wheat gluten, however.

These two preparations were then hydrolyzed and the distribution of nitrogen in the hydrolyzate was determined by the Van Slyke method, with the results shown in Table 24. Appreciable differences in ammonia (amide), cystine, arginine and lysine are apparent. The low nitrogen content (16.1 per cent), and the relatively high pH (6.45) of the isoelec-

Table 24. Distribution of Nitrogen in the Hydrolyzate of α - and β -Glutelin from Wheat Gluten, as Determined by the Van Slyke Method by Csonka and Jones.³⁸

	α -glutelin	β -glutelin
Amide N (%)	17.80	11.06
Humin N (%)	1.05	1.32
Cystine N (%)	1.76	5.43
Arginine N (%)	10.95	6.10
Histidine N (%)	5.50	6.17
Lysine N (%)	3.09	6.85
Amino N of filtrate (%)	45.40	49.13
Non-amino N of filtrate (%)	13.00	14.90

tric point of their β -glutelin convinced Csonka and Jones that it was not a globulin. The latter contains more nitrogen by the order of about 2 per cent, and commonly has an isoelectric point in the range of pH 5 to 5.5.

A more detailed study of the cystine, tryptophane and tyrosine content of the α -glutelin of wheat was made later by Csonka,³⁹ with the results recorded in Table 25. The percentages of these three amino acids in the several preparations are in good agreement, which may be partly in consequence of improvements made in the analytical methods employed.

Table 25. Amino Acids in Moisture- and Ash-free α -Glutelins from Wheat Flour, as Reported by Csonka.³⁹

α -Glutelin of	Cystine (%)	Tryptophane (%)	Tyrosine (%)
Hard wheat	1.16	2.24	5.69
Soft wheat	1.35	2.12	5.07
Commercial flour	1.44	2.04	5.30

Glutenin prepared from gluten by an adaptation of Fleurent's method was subjected to the determination of its tryptophane and tyrosine content by Tillmans, Hirsch and Stoppel.⁴⁰ Their median values were: tryptophane, 1.55 per cent, tyrosine, 7.25 per cent. This value for tryptophane is approximately double that found for gliadin extracted with cold 70 per cent alcohol.

The cystine content of the glutenin prepared by the alkali-dispersion, reprecipitation technique followed by many workers, was summarized by Neglia, Hess and Sullivan⁴¹ as follows:

³⁹ *J. Biol. Chem.*, **97**, 281 (1932).

⁴⁰ *Biochem. Z.*, **196**, 379 (1928).

⁴¹ *J. Biol. Chem.*, **125**, 183 (1938).

T. B. Osborne, <i>Carnegie Inst. Wash. Pub.</i> 84 (1907).	0.02%
O. Folin and J. M. Looney, <i>J. Biol. Chem.</i> , 83, 103 (1929).	1.80
D. B. Jones, <i>et al.</i> , <i>J. Biol. Chem.</i> , 62, 183 (1924).	1.56
R. J. Cross and R. E. Swain, <i>Ind. Eng. Chem.</i> , 16, 49 (1924).	0.91 -
	1.03
F. A. Csonka, <i>J. Biol. Chem.</i> , 97, 281 (1932).	1.16 -
	1.44
R. K. Larmour and H. R. Sallans, <i>Can. J. Research</i> , 6, 38 (1932)	
Osborne method	0.58
Blish and Sandstedt method	1.32 -
	1.42
F. J. Neglia, W. C. Hess, and M. X. Sullivan, <i>J. Biol. Chem.</i> , 125, 183 (1938).	
Acid-prepared	2.10
Alkali-prepared	1.38

The specific rotations of α - and β -glutelin of wheat in 0.5M NaOH solution were observed by Csonka, Horn and Jones.⁴² While alkali commonly effects a racemization of protein, with a consequent change in specific rotation, they found that under their working conditions the specific rotation was only slightly affected, if at all, by 0.5M NaOH at 20° up to 2 hours. Also the specific rotation was not greatly affected by variations in concentration of the glutelin, within a fairly wide range, nor was it very different in the instance of glutelins from hard and soft wheats. The specific rotation of the α -glutelin was significantly higher (-85.2°) than that of β -glutelin (-76.4°), but lower than gliadin (-112.7°) in the same solvent.

They also observed the relative racemization rates of gliadin, α - and β -glutelins in 0.5M NaOH at 38° C as registered in terms of specific rotation. Typical hyperbolic curves resulted from plotting such data against time in hours, and the three curves are of the same general character. For α -glutelin the curve is expressed by the equation: $\log (R + 158.6) = 2.3966 + (-0.0424) \log t$. For β -glutelin it becomes: $\log (R + 100) = 2.2585 - 0.0632 \log t$.

Neglia, Hess and Sullivan⁴¹ found that while the total sulfur contents of acid- and alkali-prepared glutenins^{42a} were about the same, 1.28 and 1.31 per cent respectively, the sulfur content of the hydrolyzate of the former is about the same as that of the original protein, namely 1.26 per cent, whereas the hydrolyzate of the alkali-prepared glutenin contains only 1.06 per cent of sulfur (corrected to a common basis in all cases).

The cystine content of the two sets of hydrolyzates was also determined and, as shown by the data in Table 26, there is evidence of a destruction of cystine by the alkali treatments accorded one preparation. This supports the earlier contention of Blish and Sandstedt that the acid dispersion method is to be preferred over the method involving dispersion of the protein in 0.2N NaOH.

⁴² *J. Biol. Chem.*, 89, 267 (1930).

^{42a} Blish and Sandstedt, *J. Biol. Chem.*, 85, 195 (1929).

Table 26. Cystine Content of Glutenins Corrected by Moisture and Ash, as Reported by Neglia, Hess and Sullivan.⁴¹A = HCl - HCOOH hydrolysis; B = HCl - TiCl₃ hydrolysis.

Glutenin	Sullivan method		Folin-Marenzi method		Okuda method	
	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)
Acid-prepared	2.05	2.09	2.05	2.14	2.15	2.11
Alkali-prepared	1.33	1.31	1.52	1.39	1.37	1.37

Larmour and Sallans⁴³ employed both the Osborne and the Blish and Sandstedt methods for the preparation of glutenin, with certain variations in each instance. Thus in Osborne Method II the glutenin was not dispersed in alkali after removal of gliadin from the crude gluten with 70 per cent alcohol. The preparation was less pure than in the Osborne method I, as evidenced by its low content of nitrogen, 12.23 per cent. In the instance of preparation method III, the technique described by Blish and Sandstedt in 1925¹¹ was followed, involving a variation from Osborne's method I, in that the crude gluten was dispersed directly in dilute NaOH solution, then brought to a concentration of 70 per cent alcohol and the glutenin precipitated by adding acid to its isoelectric pH. Method IV involved the use of 0.007*N* acetic in lieu of alkali in dispersing the gluten after the method of Blish and Sandstedt,^{42a} described elsewhere in this monograph. Method V varied from IV in the use of a stronger acetic acid solution, namely 0.07*N*.

All the preparations resulting from these fractionation methods were hydrolyzed and the nitrogen distribution determined by the Van Slyke method. The resulting data are recorded in Table 27. It appears that

Table 27. Nitrogen Distribution as Determined by the Van Slyke Method in the Hydrolyzate of Several Glutenin Preparations Reported by Larmour and Sallans.⁴³

	Glutenin prepared by method of—				
	I Osborne	II	III Blish and Sandstedt	IV	V
Total N in preparation (%)	16.84	12.23	16.50	17.7	16.05
Nitrogen distribution in % N					
Ammonia	12.7	18.4	15.1	21.0	16.1
Humin	2.0	2.5	1.6	1.5	2.1
Total basic	23.8	18.1	20.7	18.0	20.5
Arginine	12.6	8.6	11.8	8.5	12.1
Cystine	0.4	0.8	0.7	0.9	0.6
Histidine	7.1	4.3	3.6	1.5	3.1
Lysine	3.6	4.4	4.6	7.0	4.6
Ammonia N in filtrate	52.3	53.7	54.3	54.2	55.3
Non-amino N in filtrate	8.6	7.5	8.2	5.9	6.2

the preparations made by Method IV, using 0.007*N* acetic acid, were the purest, being highest in nitrogen, and lowest in the humin content of the hydrolyzate. There is some evidence here, as stressed by Neglia, Hess

⁴³ *Can. J. Research*, 6, 38 (1932).

and Sullivan,⁴¹ that the alkali treatment in Osborne method I reduced the cystine content below that of the acid treatment which yielded the purest gliadin (Method IV).

Specific Rotatory Power, and Racemization of Wheat Glutelins

Glutelins prepared from three types of flour were examined by Dingwall.⁴⁴ The glutenins were dispersed in 0.5*N* NaOH solution and the specific rotations were observed with monochromatic light at three wave lengths. As in the instance of the three corresponding gliadin preparations, there were no significant or consistent differences between the several glutenin preparations, as shown by the data in Table 28. Moreover, Dingwall concluded that the treatment with alkali that was involved in the preparation and observation of these glutenins had a moderate effect upon their properties. Thus the values reported by him at 5890 Å were of the same order, -74.2° to -75.3° , as reported by Woodman⁴⁶ for a relatively non-racemized preparation.

Table 28. Rotation Dispersion of Glutenin Preparations Prepared from Strong and Weak Flours in 0.5*N* NaOH, as Observed by Dingwall.⁴⁴

Wave length Angstrom	"Strong" wheat flour No. 8410	Soft wheat flour No. 8496	"Weak" cracker flour No. 8497
5500	- 60.0	- 60.4	- 59.7
5890	- 75.3	- 75.3	- 74.2
5300	- 93.4	- 93.5	- 93.3

Dingwall also determined the refractive index dispersion of the three glutenins with the results recorded in Table 29. Again, the differences between glutenins from flours of widely varying baking quality were quite similar in this property.

Table 29. Refractive Index Dispersion of Glutenins in 0.5*N* NaOH, as Determined by the Pulfrich Refractometer at 22.8° C by Dingwall.⁴⁴

Preparation	5780	Wave length (Angstrom) 5461	4358
Solvent only	1.33874	1.33976	1.34583
Flour No. 8410	1.34215	1.34317	1.34939
"a" value	0.00175	0.00175	0.00183
Flour No. 8496	1.34258	1.34361	1.34982
"a" value	0.00170	0.00170	0.00177
Flour No. 8497	1.34315	1.34420	1.35041
"a" value	0.00182	0.00182	0.00189

The "a" value of Robertson is the alteration of the refractive index of the solvent effected by dissolving 1 g of the substance in 100 cc of it.

The maximum spectrum absorption of glutenin was found by Kondo and Mituda⁴⁵ to be at 282 $m\mu$, the minimum at 272 $m\mu$. The light-absorp-

⁴⁴ "Studies of proteins of wheat flour in relation to flour strength," A thesis filed in the Library of the University of Minnesota, 1924.

⁴⁵ *J. Agr. Chem. Soc. Japan*, 16, 159 (1940); *Chem. Abs.*, 34, 4763. Original not read.

tion coefficients per nitrogen equivalent at these two wave lengths were 104.3-147.0, and 73.0-102.6 respectively, the range representing differences among the wheat varieties that were studied.

The relative rates of racemization of two glutenin preparations dispersed in 0.5*N* NaOH were traced by Woodman.⁴⁶ In the instance of the glutenin prepared from Manitoba flour, the specific rotation was -93° just one hour after making up the dispersion, and this fell to -41.5° after 240 hours. The English flour glutenin had a specific rotation of only -74° at the end of the first hour, and -32.5° after 242 hours. In a 0.25*N* NaOH solution the change was less, reaching -57° and -48.5° respectively after the 240th hour. From this, Woodman concluded that the two glutenins are unlike, *i.e.*, that they are two different proteins.

Blish and Pinckney⁴⁷ prepared glutenins from seven wheat flours of widely varying composition and baking qualities. The racemization rates of these seven glutenin preparations in 0.5*N* NaOH were observed, and, contrary to the findings of Woodman,⁴⁶ no differences were observed in the six preparations from *vulgare* wheats. The glutenin prepared from Polish wheat (*Triticum polonicum*) differed from the others, its specific rotatory power being rather uniformly lower (*i.e.*, less levorotatory), as shown by the data in Table 30. In a further discussion of the phenom-

Table 30. Specific Rotatory Power ($[\alpha]_D$) of Glutenin Preparations in Various Dispersion Media, as Reported by Blish and Pinckney.⁴⁷

Dispersion medium	(α) _D
0.5 <i>N</i> NaOH	-73.8°
0.25 <i>N</i> NaOH	-80.2°
0.1 <i>N</i> NaOH	-76.2°
0.1 <i>N</i> NaOH (Polish wheat)	-70.5°
50% acetic acid	-63.9°
" " " (Polish wheat)	-60.1°

enon of racemization Blish⁴⁸ contended that the glutenin "fractions" of Halton doubtless resulted from a previous racemization of his glutenin preparations in consequence of contact with KOH. Blish concluded that racemization must involve something more than enolization, and that some hydrolysis probably always occurs with the appearance of protein derivatives.

Substantial differences in the specific rotation of alcohol-insoluble (glutenin) fractions of the gluteins isolated from flours of different baking strengths were encountered by Halton.⁴⁹ This protein from flours of greater baking strength tended toward a higher specific rotation. Halton

⁴⁶ *J. Agr. Sci.*, 12, 231 (1922).

⁴⁷ *Cereal Chem.*, 1, 309 (1924).

⁴⁸ *Cereal Chem.*, 2, 127 (1925).

⁴⁹ *J. Agr. Sci.*, 14, 587 (1924).

also effected a fractionation of the glutenin from an alkaline solution. Normal HCl was added dropwise until a point was reached when protein separated sharply in a flocculent state. This was allowed to settle, and the supernatant liquid was siphoned off. Additional HCl was added to the latter, which became progressively more and more milky in appearance, until a point was reached when a second flocculation occurred. Both samples of precipitated protein were recovered, washed with 70 per cent alcohol, and finally dried with absolute alcohol and ether. They contained almost the same percentage of nitrogen, namely 17.38 and 17.42 per cent respectively. The first sample had a higher specific rotation, -82.5° , than the second, which was -78.1° . The racemization rates were similar, and at the end of 240 hours their specific rotations were -38.4° and -31.4° respectively. From this Halton concluded that at least two proteins were present in the alcohol-insoluble portion of gluten which differ in their optical rotation. The ratio of the fraction with high, to the fraction with low optical rotation was five to one.

In a critical study of the Halton⁴⁹ fractionation technique, Blish⁴⁸ attempted to repeat it, using five glutenin preparations from as many different sources. These were dispersed in dilute NaOH, and on addition of dilute HCl only one fraction could be obtained. In every case all the protein precipitated in the first fraction, since a negative reaction for protein was obtained when phosphotungstic acid was added to the supernatant liquid. It appeared possible that Halton's glutenin had become slightly racemized before fractionation was attempted, and this assumption received confirmation upon working with a deliberately racemized glutenin. Using this material, one protein fraction was precipitated from the alkaline dispersate on bringing it to pH 5.2 by addition of HCl, and a second fraction appeared at pH 4.4. Accordingly, Blish concluded that Halton's evidence of two glutenins was based upon mistaken evidence, and that racemization of glutenin with alkali gives rise to protein derivatives which behave differently from the unracemized protein.

Isoelectric Point of Wheat Glutelins

As in the instance of gliadin and the other cereal proteins, the determination of the isoelectric point of the glutelins cannot be made with precision or definiteness. In Table 31 a consolidation of some of the data in the literature is presented, and it is evident that the findings are not in good agreement. Since all the values here presented are based upon a fairly simple and direct method, namely the pH of flocculation from dispersions, it is conceivable that in the instance of this property, as in the studies of specific rotatory power, the pre-treatment of the protein is reflected in the results. This is suggested by the observations of Blish

et al., in which the "slightly racemized" glutenin precipitated at a lower pH than did other preparations. Possibly all the preparations were somewhat altered from the state of the native protein and hence may not represent the true isoelectric range of the unaltered protein.

Table 31. Isoelectric Point of Glutenin, as Determined by Various Investigators.

Investigators	General Method	pH
Csonka and Jones ⁵⁸	Flocculation in buffers	(α - and β -) 6.45
Blish and Sandstedt ⁵⁰	Prec. from dil. alkali	5.2 -5.4
" " "	Prec. from 0.1N NaOH in 68% ethanol	5.8 -6.0
Blish ⁴⁸	Prec. of slightly racemized glutenin in alk.	4.4
Kondo and Hayashi ⁵¹	Flocculation	5.19-5.58
Tague ⁵²	Precipitation	6.8 -7.0
Pearsall and Ewing ⁵⁸	Prec. from NaOH, or from acetic acid solns.	4.4 -4.5

Kondo and Hayashi ⁵¹ did not find any large variation in the isoelectric range or the nitrogen content of glutenins prepared from Australian, Japanese, American, Manchurian and Canadian wheat flours, although these flours varied in their content of total protein from 7.29 to 14.76 per cent. The isoelectric range of these glutenins was from pH 5.2 to 5.6 as determined from maximum flocculation, and their nitrogen content averaged 17.48 per cent.

Molecular Weight of Wheat Glutelins

At the time of estimating the minimal molecular weight of glutenin in 1925, Cohn, Hendry and Prentiss ⁵⁴ found that the information available to them depended entirely upon the analytical evidence. The estimates based upon the tryptophane, tyrosine, cystine, and β -hydroxyglutamic acid in glutenin were very consistent, as appears from the calculations recorded in Table 32.

Table 32. Minimal Molecular Weight of Glutenin, as Calculated from its Content of Certain Amino-acids by Cohn, Hendry and Prentiss.⁵⁴

Content of	Amount of constituent present (%)	Weight containing 1 molecule	Assumed number of molecules	Minimal molecular weight
Tryptophane	1.68	12,149	3	36,447
β -hydroxyglutamic acid	1.8	9,061	4	36,244
Tyrosine	4.5	4,024	9	36,216
Cystine	1.8	13,344		
"	1.56	15,397		

⁵⁰ *Cereal Chem.*, 2, 55 (1925).

⁵¹ *Mem. Coll. Agr. Kyoto Imp. Univ.*, No. 11, 1 (1931).

⁵² *J. Am. Chem. Soc.*, 47, 418 (1925).

⁵³ *Biochem. J.*, 18, 829 (1924).

⁵⁴ *J. Biol. Chem.*, 63, 721 (1925).

Chapter 4

Water- and Salt-soluble Proteins

Proteins soluble in 5 per cent K_2SO_4 solution are determined by a tentative method of the Association of Official Agricultural Chemists described in its "Book of Methods," Fifth Edition (1940) on page 217. The method involves extracting 6 g of flour with 100 ml of 5 per cent K_2SO_4 solution, shaking at 30-minute intervals for 3 hours, or agitating moderately in a shaking machine for one hour. The suspension is then allowed to settle for 30 minutes, filtered clear, and a 50 ml aliquot is Kjeldahled. The percentage of nitrogen, corrected for any nitrogen in the reagents, is multiplied by the factor 5.7 in estimating the equivalent percentage of protein.

A second tentative method is described in the same edition (page 218) which, oddly enough, is listed as a method for the determination of globulin and albumin (edestin and leucosin) and amino nitrogen. The peculiar feature of this description lies in the fact that the method which follows probably does not determine globulin and albumin as well as the method described above, which carefully avoids the use of the terms "globulin" or "albumin," but merely refers to proteins soluble in a specific solvent. A second unfortunate feature of description of this second tentative method is the inclusion of the term "edestin" to describe the globulin of flour. It appears to have been agreed by protein chemists in recent years that this term edestin would be restricted to the globulin prepared from hemp seed.

This second tentative method involves extracting 10 g of flour with 250 cc of 1 per cent NaCl solution, with occasional shaking, for 3 hours. After filtering clear, 100 ml of the filtrate is Kjeldahled. The nitrogen thus determined is corrected by deducting the nitrogen content of the filtrate of an equivalent aliquot after precipitating the proteins with phosphotungstic acid. The nitrogen of the filtrate is referred to in this method as "amino N," and this procedure will be discussed further in a later section of this monograph.

These methods do not differ materially from those employed by the earlier workers in this field, notably Teller,¹ Chamberlain,^{2, 3} Osborne,⁴ and others of that period.

¹ *Ark. Agr. Exp. Sta. Bull.* 48 (1896).

² *U. S. Dept. Agr., Bur. Chem. Bull.* 90, p. 121 (1905).

³ *J. Am. Chem. Soc.*, 28, 1657 (1906).

⁴ "The Proteins of the Wheat Kernel," Carnegie Institution of Washington, 1907.

Blish ⁵ hydrolyzed the soluble proteins in a 1 per cent NaCl extract of a strong and a weak flour, and found 12.68 and 12.84 per cent respectively, of the total nitrogen in the ammonia fraction.

Olson ⁶ concluded that 1 per cent NaCl solution extracted not only the albumin and globulin of flour, but gliadin as well. Thus about 30 per cent of the protein in such an extract appeared to be a prolamine. With 10 per cent NaCl solution as a solvent the proportion of gliadin was much smaller, being of the order of 5 per cent.

Albumin and globulin preparations were recovered from bran by Jones and Gersdorff ⁷ after several different methods. Using distilled water, a total of 3.94 per cent of protein ($N \times 6.25$) was extracted from a bran containing 17.25 per cent of protein ($N \times 6.25$). From this water extract 2.87 per cent of the bran was recovered as albumin, and 0.69 per cent as globulin, representing 16.64 and 4 per cent of the total protein in the bran, respectively. When 4 per cent NaCl solution was used as the extraction medium they recovered 1.66 per cent of the bran, or 9.62 per cent of the bran protein as globulin.

In the instance of a series of bran albumin preparations, some five in all, isolated from water-, and 4 per cent NaCl solution-extracts, the nitrogen content, on an ash- and moisture-free basis ranged from 15.09 to 15.61 per cent. Seven bran globulin preparations contained from 17.39 to 18.01 per cent N. The averages of the elementary analyses of these two proteins are recorded in Table 33.

In an extension of this study, Jones and Gersdorff ⁸ described these proteins more fully. About 2.9 per cent of their sample of wheat bran consisted of albumin, which separated as a flocculent coagulum when its aqueous (slightly acidified with acetic acid) solution was slowly heated to 60° C. In solution in 4 per cent aqueous NaCl it coagulated at about 65° C. The bran globulin was precipitated from a 4 per cent NaCl solution by the addition of $(NH_4)_2SO_4$ until 0.4-0.65 of saturation was reached. It coagulated in a slightly acidified 4 per cent NaCl solution at 95° C. The bran albumin and bran globulin both contained less nitrogen than the corresponding proteins prepared by Osborne (leucosin from flour, and embryo globulin) which contained 16.80 and 18.39 per cent of nitrogen respectively. The percentage of certain amino acids in these two proteins, together with an indication of the method employed in their determination, is given in Table 33. In general they are characterized by a high content of basic amino acids.

⁵ *J. Ind. Eng. Chem.*, **8**, 138 (1916).

⁶ *J. Ind. Eng. Chem.*, **6**, 211 (1914).

⁷ *J. Biol. Chem.*, **58**, 117 (1923).

⁸ *J. Biol. Chem.*, **64**, 241 (1925).

Table 33. Ultimate Analysis and Percentage of Certain Amino-acids in Soluble Proteins Isolated from Bran by Jones and Gersdorff,⁸ expressed as Percentages of the Proteins.

	Bran albumin %	Bran globulin %
Carbon	53.21	53.43
Hydrogen	6.71	7.40
Nitrogen	15.42	17.76
Sulfur	1.35	0.91
Oxygen	23.30	20.50
Amide N, Van Slyke method	1.20	1.06
Humin N, Van Slyke method	0.52	0.29
Basic N, Van Slyke method	5.12	7.73
Non-basic N, Van Slyke method	8.92	8.82
Cystine N, Van Slyke method	1.38	2.80
Cystine, Folin and Looney method	3.29	1.52
Arginine, Van Slyke method	10.04	14.13
Histidine, Van Slyke method	2.57	2.76
Lysine, Van Slyke method	4.51	11.84
Tyrosine, Folin and Looney method	4.20	3.69
Tryptophane, May and Rose method (modified)	4.76	2.85

Spring wheat was harvested by Sharp and Elmer⁹ at regular intervals beginning with the thirteenth day after flowering and continuing through to ripeness. The grain was milled into flour, and this was extracted with 5 per cent K_2SO_4 solution. The percentage of protein so extracted in the instance of a limited but characteristic number of samples is recorded in Table 34, based (a) upon the flour (moisture-free), and (b) upon the

Table 34. Percentage of Protein Extracted from Flour Milled from Wheats at Different Stages of Maturity, using 5 per cent K_2SO_4 as Solvent, as Reported by Sharp and Elmer.⁹

Approximate age of kernel days	Protein (N \times 5.7) extracted by 5% K_2SO_4	
	Based upon moisture- free flour (%)	Based upon total protein (%)
13	3.80	32.1
20	2.52	23.5
27	2.22	19.3
34	1.95	16.4

total protein. It is evident that not only did the percentage of soluble protein decrease in the grain, but the proportion of total protein peptized by 5 per cent K_2SO_4 decreased to about half of that encountered in the 13th day sample as the wheat approached maturity.

Water-soluble protein of wheat was purified by dialysis by Herzner¹⁰ to free it from globulins, followed by electrodialysis. The resulting preparation was free from ash, and contained 16.11 per cent nitrogen, 7.24 per cent hydrogen and 51.44 per cent carbon. Its aqueous solution had a

⁹ *Cereal Chem.*, 1, 83 (1924).¹⁰ *Biochem. Z.*, 202, 320 (1928).

reaction of pH 6.69; electrophoresis tests showed the amphoteric range to be about pH 5.10. Maximum heat coagulation occurred at about pH 4.97.

Two globulin preparations were recovered from wheat flour by Hoffman and Gortner.¹¹ Globulin "A" was made by extracting with 5 per cent K_2SO_4 solution in the ratio of 1 kilo of flour to 3 liters of solution. After shaking and allowing to settle, the extract was clarified through a supercentrifuge, dialyzed in Cellophane until free from sulfates, and the precipitated globulin recovered in a supercentrifuge. This material was essentially insoluble in 5 per cent K_2SO_4 , and in 10 per cent NaCl solutions. It was dissolved in a small volume of 0.2 per cent NaOH, precipitated by neutralizing with HCl, and the precipitated globulin recovered by filtering, and washed with 70 per cent ethanol, absolute ethanol and anhydrous ether.

Globulin "B" was extracted with 10 per cent NaCl. The dispersate was viscous and the flour particles settled out slowly, even with a ratio of 1 kilo of flour to 6 liters of solution. After centrifuging and dialyzing, the precipitated "globulin B" proved to be soluble in 10 per cent NaCl, and was redissolved and reprecipitated by dialysis. It was then recovered, washed with 70 per cent ethanol, absolute ethanol and ether. Its nitrogen content was about $3\frac{1}{2}$ per cent higher than globulin "A." The fractionation by the Van Slyke procedure of the products of acid hydrolysis of globulins "A" and "B" is shown in Table 35.

Albumin "A" was recovered from the 5 per cent K_2SO_4 extract from which the globulin "A" had been precipitated by dialysis. The solution from the dialyzate was concentrated *in vacuo* at $<50^\circ C$ and the albumin precipitated by half saturation with $(NH_4)_2SO_4$. This precipitate was recovered in a supercentrifuge and washed again with half-saturated $(NH_4)_2SO_4$ solution. It was then dissolved in water, and reprecipitated by half saturation with $(NH_4)_2SO_4$. The precipitated globulin "A" was washed with more $(NH_4)_2SO_4$, and then redissolved in water, and dialyzed in Cellophane until free of sulfates. At this stage the albumin in solution was not heat-coagulable until a trace of acid was added. The albumin "A" was then precipitated by pouring the aqueous solution into strong alcohol, the albumin recovered by filtration, washed with ethanol, followed by ether, and dried. A yield of 21 grams, having a dark-brown appearance, was recovered from the original 18 kilos of flour.

Albumin "B" was recovered in an essentially similar manner from the solution remaining after the dialysis and precipitation of globulin

¹¹ *Cereal Chem.*, 4, 221 (1927).

"B" from the 10 per cent NaCl extract of flour. The yield was very small; only $1 \pm$ g from 14 kilos of flour. The Van Slyke fractionation of the products of acid hydrolysis of this, and albumin "A" is shown in Table 35.

Table 35. Nitrogen Distribution in the Products of Hydrolysis of "Soluble" Wheat Proteins after the Method of Van Slyke, as Reported by Hoffman and Gortner.¹¹

	Albumins		Globulins	
	A %	B* %	A %	B† %
Ammonia N	10.67	10.28	8.28	10.60
Humin, acid-insol. N	0.93	1.14	2.13	0.59
acid-sol. N	1.13	2.59	1.26	0.49
Histidine N	4.44	4.63	1.29	11.27
Cystine N	1.63	1.87	2.39	2.04
Arginine N	15.70	10.69	14.29	18.64
Lysine N	9.50	4.81	6.84	2.39
Filtrate N				
mono-amino	49.47	50.84	55.91	42.42
non-amino	7.04	17.01	7.87	11.42
Total N in protein	16.73	13.51*	14.77	18.26

* Air-dry basis.

† Ash- and moisture-free basis.

Attention was called by Gortner, Hoffman and Sinclair¹² to the inadequacy of the definition of *globulin* adopted by the Physiological and Biochemical Committees on Protein Nomenclature^{13, 14} which reads, "simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases. (The precipitation limits with ammonium sulfates should not be made a basis for distinguishing the albumins from the globulin.)" Even with the addition of the adjective "dilute" in describing the saline solutions, as has been made in many text-books, the definition still leaves the inference that all salts of strong acids with strong bases are equally suitable for extracting globulins.

These investigators proceeded to extract 12 flour samples with aqueous solutions of 18 salts at several levels of concentration, including 0.5N, 1.0N, 2.0N, and 5 per cent or 10 per cent. The detailed data in terms of the percentage of total protein extracted from each flour by these 72 solutions are recorded in extensive tables. A convenient consolidation of certain of these data appear in Figure 1, which records graphically the average percentage of protein extracted at three or more levels of salt concentration from the 12 flours. It is evident that the concentration of salt in the solvent was not very significant within the limits of 0.5 to 2.0N in the instance of many of the salts, such, for example, as KF, Na₂SO₄, MgSO₄, Na₃ citrate, Na₂HPO₄, and LiCl. With a few salts, including

¹² *Cereal Chem.*, 6, 1 (1929).

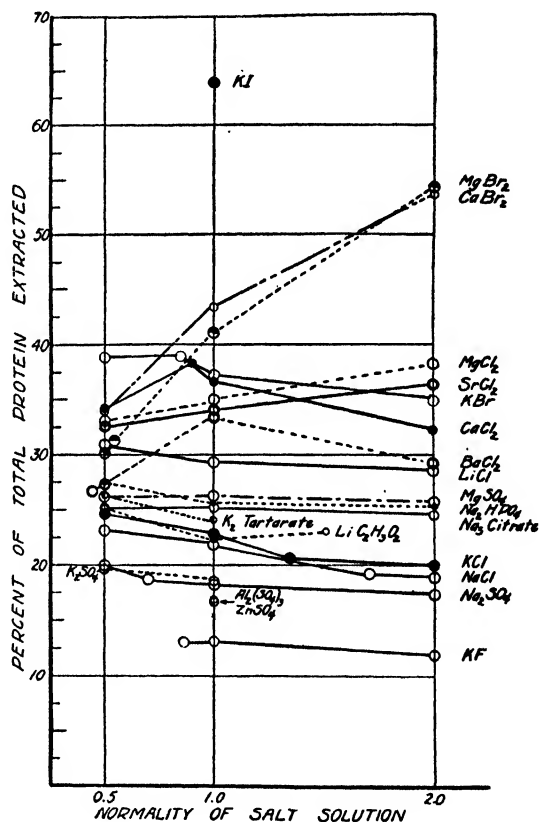
¹³ *J. Biol. Chem.*, 4, xlviii (1908).

¹⁴ *Am. J. Physiol.*, 21, xxvii (1908).

KCl, NaCl, K_2 tartrate, there appeared to be a slight trend toward a decrease in the proportion of protein extracted or increasing the concentration of salt in the saline solvent. Again, a number of salt solutions effected a progressively increasing extraction of protein with increasing concentration, slightly with $MgCl_2$ and $SrCl_2$, and very decidedly with $MgBr_2$ and $CaBr_2$.

A glance at the chart reveals the wide range in peptization of the flour proteins with the several saline solutions, extending from 11.9 per cent of the protein with 2N KF, to 63.9 per cent with 1N KI. A lyotropic or

FIG. 1. Average percentage of protein extracted from twelve wheat flours by various concentrations of salt solutions as reported by Gortner, Hoffman and Sinclair.¹²



Hofmeister series of increasing peptization appeared in the instance of the anions as: $F' < SO_4' < Cl' < tartrate'' < Br' < I'$; in the instance of the cations as: $Na' < K' < Li' < Ba'' < Sr'' < Mg'' < Ca''$. It thus appeared that protein "solubility" in neutral salt solutions is in reality protein peptization, governed as to rate and extent by the nature of the cations and anions present in the salt solution. It seemed doubtful as to whether or

not any of these saline solutions extracted a chemical entity which could be termed a globulin.

Also it was noted that the averages expressed in the graphs in Figure 1 do not maintain uniformly in all flours, but, as a matter of fact, there was considerable variability about the mean.

Certain of these observations of Gortner, Hoffman and Sinclair were confirmed by Herd.¹⁵

Wheat meal was prepared by grinding whole wheat grains of different varieties of *Triticum vulgare* and other *T.* species, and then extracted with half-molar solutions of four different potassium halides, and K_2SO_4 , as well as distilled water, in the studies of Staker and Gortner.¹⁶ The composition of the meal, and the percentage of the total nitrogen extracted by the six solvents is shown in Table 36. It is apparent that the proportion of the total protein extracted increases fairly regularly with the series $F' < SO_4^{2-} < Cl' < Br' < I'$ in the instance of these potassium salts, at the 0.5M concentration, and all of them extracted more than water. There was fairly good agreement in terms of the proportion of the protein that was extracted by an individual salt in the case of these meals, except in the instance of the variety Quality. The variety Marquis, also of the species *T. vulgare*, was second in order, but oddly enough, it seemed to resemble less closely related species more than it resembled Quality.

Staker and Gortner then expanded this line of investigation to include an effort to estimate the amount, and ratios of albumin and globulin in these wheat meals. The basis of their estimates is clearly indicated in the column heads of Table 37, where their data are also recorded. The estimated albumin (as percentage of the total protein), is shown in Column B, that of globulin in Column E. The latter is several times as great as the albumin, the maximum ratio being reached with spelt, Quality, and Einkorn. Using the average values in this and the preceding table it would appear that these wheats contained a mean of 0.69 per cent of albumin ($N \times 5.7$) and about 3.93 per cent of globulin. The sum of these, or 29.4 per cent of the total protein, is slightly more than the percentage of total protein peptized by K_2SO_4 solution, which was 26.2 per cent, and slightly less than was peptized by 0.5M KCl, which was 31 per cent.

Certain of the data of Gortner, Hoffman and Sinclair¹² were subjected to statistical analysis by Geddes and Goulden.¹⁷ They found a high negative correlation of $r = -0.83$ between the percentage of total pro-

¹⁵ *Cereal Chem.*, 8, 1 (1931).

¹⁶ *J. Phys. Chem.*, 35, 1565-1605 (1931).

¹⁷ *Cereal Chem.*, 7, 527 (1930).

Table 36. The Percentage of the Total Nitrogen Peptized from Various Wheat Meals by Distilled Water and by 0.5M Salt Solutions, as Reported by Staker and Gortner.¹⁶

Wheat Species	Wheat Variety	Moisture %	Ash %	Total Nitrogen %	Total Nitrogen Peptized by			
					H ₂ O %	KF %	K ₂ SO ₄ %	KCl %
<i>T. turgidum</i>	Alaska	9.73	1.96	2.729	19.58	21.86	23.74	28.29
<i>T. monococcum</i>	Ein Korn	9.37	2.22	2.891	17.89	22.39	23.27	27.67
<i>T. dicoccum</i>	Emmer	9.32	2.25	2.477	21.28	21.08	22.65	28.33
<i>T. compactum</i>	Little Club	10.57	2.25	2.99	19.72	25.97	27.10	30.61
<i>T. vulgare</i>	Marquis	10.50	1.69	2.464	23.16	26.84	28.63	33.24
<i>T. durum</i>	Mindum	10.47	2.25	3.017	21.58	24.93	26.01	30.91
<i>T. polonicum</i>	Polish	8.34	1.81	3.64	18.34	21.97	23.20	27.39
<i>T. vulgare</i>	Quality	9.58	2.11	2.112	26.72	32.74	34.59	40.53
<i>T. spelta</i>	Spelt	9.18	2.28	2.509	23.26	25.94	26.93	31.83
	Average	9.67	2.09	2.76	21.28	24.86	26.24	30.98
								40.16
								55.01

Table 37. A Comparison of the Various Nitrogen Fractions Expressed as Per Cent of the Total Nitrogen and the Albumin: Globulin Ratio Obtained from the Extracts of Wheat Meals as Reported by Staker and Gortner.¹⁶

Wheat Variety	Total Nitrogen peptized by—				Globulins extracted by Water A-B=D %	Total Globulin C+D=E %	Total Albumin + Globulin B+E=F %	Extraction originally by 0.5M KCl (No water Extraction) G %	Globulin denatured by dialysis which then appears in the Glutelin H %	Globulin Albumin D/B
	Water* (Albumins + Some Globulin in Soln.) A %	Water Following Dialysis (Albumins in Soln.) B %	0.5M KCl Following Water Extractions (Globulin) C %	D %						
Alaska	19.18	4.70	8.71	14.48	23.19	27.89	28.29	0.40	0.09	3.08
Ein Korn	16.67	2.77	10.91	13.90	24.81	27.58	27.67	0.09	0.09	5.02
Emmer	20.39	5.00	8.29	15.39	23.68	28.68	28.33	-0.35	0.35	3.08
Little Club	18.94	4.01	10.05	14.93	24.98	28.99	30.61	1.62	1.62	3.72
Marquis	21.21	7.04	8.08	14.17	22.25	29.29	33.24	3.95	3.95	2.01
Mindum	19.34	5.94	8.57	13.40	21.97	27.91	30.91	3.00	3.00	2.26
Polish	17.47	3.74	8.54	13.73	22.27	26.01	27.39	1.38	1.38	3.67
Quality	24.94	3.78	13.01	21.16	34.17	37.95	40.53	2.58	2.58	5.60
Spelt	21.92	2.69	8.42	19.23	27.65	30.34	31.83	1.49	1.49	7.15
Average	20.01	4.41	9.40	15.60	25.00	29.41	30.98	1.57	1.57	3.53

*The values in this column differ somewhat from values in the similar column of Table 36, because a different technique was employed. In Table 36 the data represent the extractions of six-gram samples with three successive treatments of 50 cc. of water, each treatment with water being for 30 minutes. In the present table a *single* extraction with 200 cc. of water during a two-hour period was employed.

tein in flour, and the percentage of total protein peptized by 0.5*N* MgSO₄ solution. This may be interpreted to mean that those proteins "soluble" in or peptized by the solution employed did not increase in percentage with increasing total protein content. This raised the issue as to whether or not this resulted from a trend toward decreasingly complete extraction of these soluble proteins as the total protein content increased. To satisfy themselves on this point, Geddes and Goulden extracted eight flours ranging in total protein content from 8.6 to 13.7 per cent, with six successive portions of 0.5*N* MgSO₄ solution. The last three extracts of each flour were combined and Kjeldahled, and it appeared that the protein in these extracts was uniformly small in amount. This shows that the original, single extractions were reasonably uniform in terms of the completeness of extraction; thus the high negative correlation indicated above could not be attributed to incomplete extraction.

A series of solvents including water, 0.5*N* KF, 0.5*N* KCl, 0.5*N* KBr and 0.5*N* KI were applied to five flours, ranging in protein content from 8.0 to 18.3 per cent of total protein, by Harris.¹⁸ When the protein extracted was calculated in terms of the percentage of total protein, there was a decrease in this percentage with increasing total protein content. While 0.5*N* KF extracted slightly less protein than water, there was a steady increase in the extracted protein in progressing through the remainder of the series of solvents. The averages for the 5 flours were: Water, 19.2 per cent; 0.5*N* KF, 15.4 per cent; 0.5*N* KCl, 21.5 per cent; 0.5*N* KBr, 32.6 per cent; 0.5*N* KI, 47.1 per cent. In this same study it was observed further that the quantity of protein extracted was about the same at 20, 38 and 58° C when the temperature of extraction was varied stepwise through this range. Also the extraction was quite incomplete in 5 seconds, increased sharply up to 15 minutes, and slowly thereafter up to 60 minutes.

Twenty streams of flour from a roller mill were analyzed by Harris¹⁹ and subjected to baking tests. Although his data were not subjected to statistical analysis in respect of the relation between ash content and percentage of protein peptized by the several solvents, scanning the data suggests a positive correlation. A few typical relationships have been selected to illustrate:

Stream	Ash (%)	Protein peptized (as % of flour) by 0.5 <i>N</i> MgSO ₄ 0.5 <i>N</i> KBr	
3rd middlings	0.34	1.95	3.25
5th middlings	0.47	2.20	3.97
6th middlings	0.61	2.51	3.93
5th break	0.83	3.74	4.88
Reel No. 4	1.92	6.67	7.40

¹⁸ *Cereal Chem.*, 8, 47 (1931).

¹⁹ *Cereal Chem.*, 8, 113 (1931).

A somewhat similar study of mill streams was made by Pascoe, Gortner and Sherwood.²⁰ While the ash content of the streams was not recorded, they also reported a large range in the relative amount of protein peptized by each of the four solvents which were applied to their 17 flours. Their data were recorded as percentage of the total nitrogen peptized by the solvent. The averages of all the flours were: 0.5N KBr, 28.31 per cent; 0.5N KCl, 21.23 per cent; 0.5N KF, 16.31 per cent; and 0.5N MgSO₄, 20.07 per cent. These are in the same order and of about the same magnitude as the observations of Harris.¹⁹ Without a single reversal, the ion effect in the series of Pascoe *et al.* was Br' > Cl' > F', in terms of the proportion of protein peptized. Pascoe *et al.* also computed the coefficient of variability of the peptized protein data in the instance of the four solvents employed by them; for the 17 mill streams it was: KBr, 12.6 per cent; KCl, 18.9 per cent; KF, 24.5 per cent; MgSO₄, 18.9 per cent. It thus appears that 0.5N KF solution effected the largest variability in proportion of protein peptized, although it invariably peptized the least protein of the four solvents. Conversely, KBr, which peptized the most protein, tended to peptize more nearly constant proportions. While in this series of flours there was a significant correlation between the nitrogen content of the flour and the amount of nitrogen peptized, the average for the four solvents being $r = 0.859$, with small differences between them, it should be emphasized that this relation may not be expected to maintain if such comparisons are restricted to flours of the same grade, but of varying protein content.

When wheat meal was extracted with the two solvents 0.5N MgSO₄, and 0.5N KBr, Harris²¹ found less difference in the percentage of the total protein peptized than when the same two solvents were applied to the flours milled from these wheats. In the instance of a series of 15 wheats, and the corresponding flours, the averages were as follows:

	% total protein peptized by	
	0.5N MgSO ₄	0.5N KBr
Flour	23.9	30.9
Wheat	17.1	28.7

A further extension of this series of studies by Harris²² included 0.5N NaCl, together with MgSO₄ and KBr and water as a peptizing medium. When these three solvents were applied to a collection of 15 flours, the average percentages of the protein present that were extracted were as follows:

²⁰ *Cereal Chem.*, 7, 195 (1930).

²¹ *Cereal Chem.*, 8, 190 (1931).

²² *Cereal Chem.*, 9, 147 (1932).

Water	21.5%
0.5 <i>N</i> MgSO ₄	15.6%
0.5 <i>N</i> NaCl	19.7%
0.5 <i>N</i> KBr	26.9%

Varietal differences in the peptizability of flour proteins by saline solutions was not large in the instance of the wheats examined by Mangels.²³ A trend toward a negative correlation of total nitrogen content with the nitrogen in the proteins peptized by *N* K₂SO₄ and *N* MgCl₂ solutions was observed, although the negative value for the coefficient of correlation *r* was small and only slightly greater than the probable error of the constant. There was a regular and substantial increase in the proportion of the total protein peptized by *N* K₂SO₄, *N* MgCl₂ and *N* KBr respectively, the ratios being of the approximate values of 1:2:2½.

Albumin and globulin were extracted from wheat and wheat products by Teller and Teller²⁴ with 5 per cent NaCl solution. The albumin was separated by heating to 95° C for an hour, collecting the coagulum and determining the nitrogen in it by Kjeldahling. The globulin was precipitated from an aliquot of the saline extract by adding four volumes of U. S. No. 30 alcohol, Kjeldahling the coagulum, and then deducting from the nitrogen thus determined the nitrogen of the albumin, as recovered by heat-coagulation. The difference was recorded as globulin nitrogen. In Table 38 appear the data resulting from such a fractionation of wheats, brans, and flours. It is evident that the pericarp of wheat, prominently

Table 38. Albumin and Globulin Nitrogen, as Percentage of the Total Nitrogen in Wheats, Flours, and Brans, as Determined by Teller and Teller.²⁴

Material	Total Nitrogen (%)	Albumin N percentage of total nitrogen	Globulin N
Spring wheat	3.10	8.7	3.6
Spring wheat flour	2.92	5.8	3.1
Spring wheat bran	3.12	22.4	16.0
Soft winter wheat	2.28	8.7	10.5
Soft winter wheat flour	1.91	5.2	5.2
Soft winter wheat bran	2.53	19.0	16.6

represented in the bran, must contain a substantially larger proportion of its total protein in the form of albumin and globulin than does the endosperm.

A high correlation between ash content and the quantity of protein peptized by various saline solutions was noted by Rich.²⁵ He recalculated the data of Gortner, Hoffman, and Sinclair,¹² and of Harris^{26, 27} and found that with solutions of KF, Na₂SO₄, KCl, and MgSO₄ the co-

²³ *Cereal Chem.*, 11, 164 (1934).

²⁴ *Cereal Chem.*, 9, 560 (1932).

²⁵ *Cereal Chem.*, 10, 222 (1933).

²⁶ *Cereal Chem.*, 8, 113 (1930).

²⁷ *Ibid.*, 8, 190 (1930).

efficient of correlation (r) between protein peptized and ash content of the flour exceeded the 5 per cent in the former's researches, while the same was true with MgSO_4 and KBr in the data of Harris. In his own investigations, using three series of flours in which the total range of ash content was from 0.34 to 2.30 per cent, a high correlation was observed between the percentage of ash and that of protein peptized by normal MgSO_4 solution. The coefficients of correlation (r) for the three series were: Mill A, crop of 1930, $r = + 0.933$; for Mill B, crop of 1930, $r = + 0.937$; and for Mill A, 1931 crop, $r = + 0.996$, with the 5 per cent point for r at $+ 0.349$.

The graphs based upon the two crops did not superimpose, however, the 1931 crop disclosing a greater tendency to peptize at every different level of ash content than did the 1930 crop. On computing the regression lines for these two series the following equations resulted:

$$\text{1930 crop, } y = 2.00 + 2.3 (x - 0.50)$$

$$\text{1931 crop, } y = 2.40 + 3.0 (x - 0.50)$$

where y represents the percentage of peptized protein, and x the ash content.

Rich suggested that the earlier investigations on the influence of phosphatides upon flour properties may provide an explanation for the relationship of ash content and protein peptizability. Presumably he assumes that the phosphatide content, or the magnitude of its effect, progresses regularly with the ash content. He also emphasized that the quantity of protein peptized by saline solvents is independent of the total protein content.

Water, as a solvent, did not effect a regular increase in the quantity of protein extracted from a series of flours of progressively increasing ash content, in Rich's²⁸ experiments, however. This suggested that the higher content of mineral-salt ions present in the aqueous suspension of high ash flours might depress the "solubility" of the proteins present. In one series of flours in which the ash content ranged from 0.34 to 0.95 per cent, the percentage of total protein extracted by water alone started at 25 per cent with the low-ash flour, decreased to 13.8 per cent at 0.54 to 0.72 per cent ash, and then increased somewhat, to 14.3 per cent at 0.95 per cent ash. Likewise, an increasing concentration of certain salts, including NaCl , MgSO_4 , and Na_2SO_4 , effected a reduced dispersion of protein present, with the greatest proportional effect registered with the low-ash flour. These observations cast further doubt on the value of an attempt to characterize the proteins of flour on the basis of their "solubility" in

²⁸ *Cereal Chem.*, 13, 522 (1936).

various solvents, an issue raised so definitely by Gortner, Hoffman and Sinclair.¹² Moreover, there is the further issue of whether one can depend upon a single extraction, and if multiple extractions are employed, when is the extraction complete?

In an effort to find a satisfactory method of identifying rye flour in mixture with wheat flour, König and Bartschat²⁹ observed that about half of the protein of rye is dispersed by an aqueous solution of CaSO_4 of a concentration of 0.22 g in 100 cc, while only 28 per cent of the total protein of wheat was so dispersed. Of a considerable number of solvents that were tried, this gypsum water gave the most distinctive differences between flours from the two cereals in question.

As in various other studies, they also found that 50 per cent ethanol extracted more protein (55 per cent of the total) from wheat flour than did other ethanol concentrations. With methanol, a maximum of 44 per cent of the total protein was extracted with 70 per cent methanol; with acetone a maximum of 55 per cent was extracted with 40 per cent acetone.

The percentage of the total nitrogen present in a series of extracts of hard and soft wheats was determined by Csonka.³⁰ The defatted wheat meal was first extracted with 1 per cent NaCl solution, using three successive portions for 1 hour each and at 6°-8°. Then three extractions with 60 per cent ethanol followed, the first two for 1½-2 hours, and the third over night. The next treatment of the residue was with acid and alcohol. First, 100 cc of cold HCl solution was mixed with the meal, to which was added 150 cc of 95 per cent ethanol with constant stirring. Then the mixture was centrifuged and the liquid set aside. The treatment was repeated and the two extracts combined. Finally, the nitrogen content of the residue was determined. The percentage of the total nitrogen present in these four fractions is recorded in Table 39.

Table 39. Nitrogen Extracted by Various Solvents from Three Wheat Meals, Recorded as Percentage of Total Nitrogen by Csonka.³⁰

	Wheat varieties		
	Tenmarq	Marquis	Fulhio
1% NaCl	24.4	24.0	26.8
60% ethanol	32.0	38.5	32.1
Acid-alcohol	20.5	14.5	15.8
Residue	18.8	15.0	19.2

When flours were extracted with 0.5N solutions of the alkali halides by Krejci and Svedberg,³¹ the molecular weights of the proteins in the extract, as determined from sedimentation velocity in the ultracentrifuge, were

²⁹ *Z. Untersuch. Lebensm.*, 46, 321 (1923).

³⁰ *J. Biol. Chem.*, 118, 147 (1937).

³¹ *J. Am. Chem. Soc.*, 57, 1365 (1935).

found to increase in the order KF, KCl, KBr and KI. When extracts of flour made with 0.5*N* solutions of KF and KCl were fractionated by dialysis, the material precipitated consisted largely of gliadin and globulin. The globulin, which ordinarily had a sedimentation constant of 11, polymerized in concentrated solution to molecules of sedimentation constant 17 and 25, and dissociated on dilution.

The isoelectric point of leucosin has been observed rather generally to lie in a more acid range than in the instance of the gluten proteins. In fact, this has been observed commonly in the instance of the albumins. Luers and Landauer³² found it to be in the range pH = 4.6; Pearsall and Ewing³³ employed a precipitation method and reported pH = 4.5; Csonka, Murphy and Jones³⁴ flocculated it from buffer solutions, using albumin derived from bran, and recorded a somewhat more acid isoelec-

Table 40. Nitrogen Distribution, as Determined by the Van Slyke Method in the Hydrolyzate of Several Albumin Preparations from Wheat Flour and Bran, as Reported by Various Investigators.

	Hoffman and Gortner ¹¹		Jones and Gersdorff ⁸
	A	B	Bran
Ammonia N	10.67	10.28	7.66
Humin N, total	2.06	3.73	3.31
Arginine N	15.70	10.69	20.70
Cystine N	1.63	1.87	2.10
Histidine N	4.44	4.63	4.45
Lysine N	9.50	4.81	5.54
Amino N in filtrate	49.47	50.84	56.28
Non-amino N in filtrate	7.04	17.01	0.80

Table 41. Nitrogen Distribution, as Determined by the Van Slyke Method in the Hydrolyzate of Several Globulin Preparations from Wheat Flour and Bran, as Reported by Various Investigators.

	Hoffman and Gortner ¹¹		Jones and Gersdorff ⁸
	A	B	Bran
Ammonia N	8.28	10.60	5.89
Humin N, total	3.39	1.08	1.59
Arginine N	14.29	18.64	25.25
Cystine N	2.39	2.04	0.89
Histidine N	1.29	11.27	4.15
Lysine N	6.84	2.39	12.60
Amino N in filtrate	55.91	42.42	46.14
Non-amino N in filtrate	7.87	11.42	2.84

tric point, *viz.*, pH 4.2. They also reported upon a bran globulin, which had an isoelectric point of pH 5.5.

For convenience, the results of the studies of the nitrogen distribution in the products of acid hydrolysis, as determined by the Van Slyke method by several investigators, are recorded in Table 40 for leucosin, and in

³² *Z. Elektrochem.*, **28**, 341 (1922).

³³ *Biochem. J.*, **18**, 329 (1924).

³⁴ *J. Am. Chem. Soc.*, **48**, 763 (1926).

Table 41 for globulin. A comparison of these values with those reported for gliadin and glutenin recorded in earlier chapters shows several striking differences. These "soluble" non-gluten proteins yield less ammonia ("amide") nitrogen on hydrolysis. Their lysine content is higher, particularly in comparison with gliadin. The arginine content is also high.

Tryptophane and tyrosine contents of water- and salt-soluble proteins of flour were determined by a novel method developed by Tillmans, Hirsch and Stoppel,³⁹ with the following results:

	Water-soluble protein	Protein extracted by 10% Na ₂ SO ₄ sol.
Tryptophane (%)	1.54	2.58
Tyrosine (%)	1.53	1.18

While the tryptophane content of these two proteins is substantially higher than that of the gliadin extracted with cold 70 per cent ethanol (0.79 per cent), the tyrosine content is much lower than the values reported for gliadin (5.2 per cent).

Methionine in bran globulin and bran albumin was not present in as large proportions as cystine, according to Jones.⁴⁰ His data are as follows:

	Cystine (%)	Methionine (%)
Bran globulin	0.66	0.26
Bran albumin	2.00	1.19

The sum of these two sulfur-containing amino-acids is appreciably higher in the albumin than in the globulin preparation.

From a petroleum-ether extract of flour, Balls, Hale and Harris⁴¹ obtained a protein-like substance. The extract was hydrolyzed in the cold with hydrochloric acid, and the precipitate formed was washed with absolute alcohol and ether. It was soluble in water and dilute alcohol but not in fat solvents; from aqueous solutions it could be salted out by ammonium sulfate. After three crystallizations it was found to contain 17.35 per cent of nitrogen, 4.42 per cent of sulfur, 6.56 per cent of chlorine, and no detectable phosphorus or selenium. The hydrolyzate contained 20.4 per cent of arginine, 15.7 per cent of cystine, 3.0 per cent of tyrosine, and 1.65 per cent of amino nitrogen, which fractions represented 37.8, 10.5, 1.3, and 9.5 per cent of the total nitrogen respectively. Apparently all the sulfur existed as cystine. This distinguishes it from the known protamines which do not contain cystine, although they contain much arginine, as does this protein from flour. The molecular weight of the latter (about 12,000) is greater than that of gramicidin (1,400) and of

³⁹ *Biochem. Z.*, **198**, 379 (1928).

⁴⁰ *Cereal Chem.*, **14**, 771 (1937).

⁴¹ *Cereal Chem.*, **19**, 279 (1942).

tyrocidin. The wheat protein under study probably exists in flour combined with a phosphorus-bearing lipoid, and therefore may be a component of one member of the little understood class of lipo proteins.

This crystalline protein was observed by Stuart and Harris ⁴² to have bactericidal and fungicidal properties. The latter was manifested when tested against bakers' yeast, *Saccharomyces cerevisiae*, as well as other strains of yeast and certain pathogenic fungi, and was active in high dilution.

Further studies of the crystalline protein by Balls, Hale and Harris ⁴³ disclosed that it is on the borderline between true proteins and their degradation products. Analytical data show that its molecular weight is at least 6,000, and measurements of the rate of diffusion indicate a value of about 10,000, although the method is not precise. In view of its high sulfur content and of its source, the name "purothionin" was proposed.

Evidence based upon its digestibility with certain proteolytic enzymes which attack the CO-NH linkages led to the conclusion that peptide linkages between the amino acids are common.

⁴² *Cereal Chem.*, **19**, 288 (1942).

⁴³ *Cereal Chem.*, **19**, 840 (1942).

Chapter 5

Crude Protein and Crude Gluten

If Osborne's elementary analyses of the four principal flour proteins, albumin, globulin, gliadin, and glutenin, are applied to the ratio of these proteins in flour as reported by him, the weighted mean of the nitrogen factor would become $N \times 5.69$. However, this factor was not accepted at once by all the earlier investigators in converting nitrogen content into protein equivalent. This was chiefly because the factor $N \times 6.25$ had been applied to feeding stuffs of plant origin, and it was confusing to use one factor for such mill feeds as bran and shorts, or even ground wheat itself when used as feed, and another factor for the flour, or for wheat when used for human food. Obviously the protein content of the products of milling would not total 100 per cent of the protein in the wheat used if two factors were employed in converting nitrogen content of the several mill products into equivalents of protein content. Also the issue might, and for that matter still does arise, as to when certain mill streams cease to be classifiable as potential flour streams and become component parts of some feed product.

As late as 1909 Ladd, as associate referee of the Association of Official Agricultural Chemists, applied the factor $N \times 6.25$ to the conversion of the nitrogen content of flour proteins, collectively and individually, into terms of protein content. Even later than that date he still employed the same factor in recording wheat and flour protein data in bulletins of the North Dakota Agricultural Experiment Station.

Kosutany¹ tabulated the results of analyses of several hundred Hungarian wheat samples and employed the factor $N \times 6.25$ (see p. 72 of his book) to the Kjeldahl nitrogen results, in recording the crude protein data.

Accordingly, in reading the earlier literature it is necessary to note the nitrogen factor, if recorded, with the probability in mind that, prior to 1910 at least, many of the tabulations of protein data are based upon the factor $N \times 6.25$, or some factor other than $N \times 5.7$.

Gradually the swing toward the factor $N \times 5.7$ set in, however, and in "Methods of Analysis," published by the Association of Official Agricultural Chemists in 1920, the factor $N \times 5.7$ is recorded as official for

¹ "Der ungarische Weizen und das ungarische Mehl," Budapest, 1907. (Note pp. 102-108).

(total) protein. It appears also in the tentative method for the determination of glutenin, although, oddly enough, no nitrogen factor is included in the description of the tentative method for alcohol-soluble proteins, nor for the soluble proteins of flour. Nor was this omission rectified in the 1925 edition of the same book.

"Methods of Analysis of Cereals and Cereal Products," published by the American Association of Cereal Chemists (1928) does include the nitrogen conversion factors for the flour proteins uniformly as $N \times 5.7$.

The incorrectness of this factor as applied to the mixture of proteins in whole wheat was emphasized by Jones.² As has been indicated elsewhere in this monograph, the proteins of bran are not identical in their properties, including their nitrogen content, with analogous proteins of the endosperm of wheat. Thus Jones found the following percentages of nitrogen in the three prominent bran proteins, and the amount of protein indicated in the last column was isolated from each 100 g of bran:

Protein	Nitrogen (%)	Amount isolated for 100 g bran
Albumin	15.4	2.87
Globulin	17.7	2.35
Prolamin	15.3	5.35
		<hr/> 10.57

On the basis of these data, Jones computed that these bran proteins collectively contain 15.86 per cent of nitrogen, corresponding to the conversion factor $N \times 6.31$.

In an extension of these calculations Jones found that the weighted percentage of nitrogen in all the proteins of the several structures of the wheat kernel would result in these conversion factors, namely: bran, $N \times 6.31$; endosperm, $N \times 5.70$; embryo, $N \times 5.80$. Fitting all the data into an over-all computation, the proteins of the entire kernel would appear to contain 17.161 per cent of nitrogen, and the correct conversion factor would become $N \times 5.83$.

This calculation of Jones was published at a time when large quantities of wheat were being merchandized on the basis of protein content, and when protein premiums were ranging around 10 cents per per cent of protein. The implied proposal of a new protein factor created a great deal of discussion, particularly within the grain trade. If official recognition had been taken of it promptly, wheat then in storage could have been delivered as having a higher percentage of protein than it was presumed to contain when first purchased and placed in storage, unless contracts involving protein premiums were restated in terms of nitrogen, rather than protein content. This restatement would have been confus-

² *Cereal Chem.*, 3, 194 (1928).

ing at the time, since the dealers in wheat had become familiar with protein percentages and would have been obliged to acquaint themselves with a new basis. Finally, it appeared to be tacitly agreed in both official and industrial circles that a change in the protein factor for wheat would be unwise, even though greater scientific accuracy might result; accordingly, the factor $N \times 5.7$ is still applied generally in the United States in determining the percentage of protein in both wheat and flour.

It has long been known that the percentage of protein in wheat is highly variable, particularly if the comparisons are extended to include

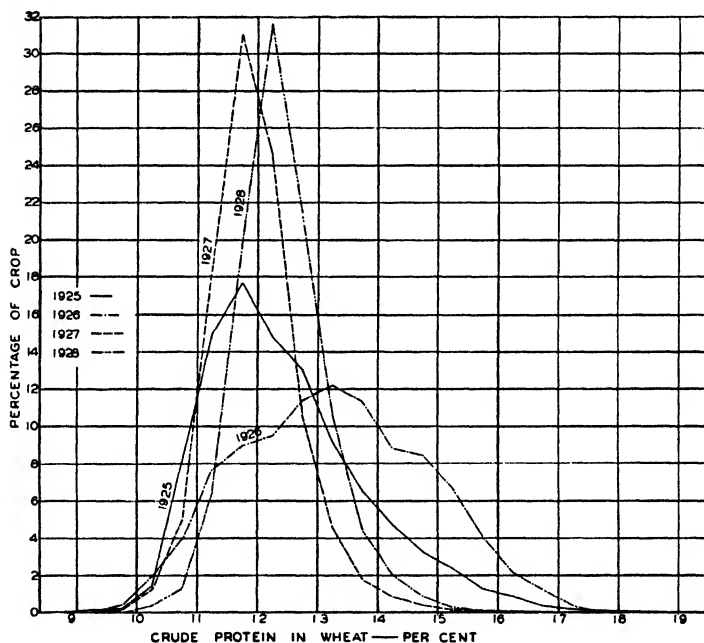


FIG. 2. Distribution curves showing the percentages of all hard spring wheat, crops of 1925, 1926, 1927, and 1928 marketed through Minneapolis, Minnesota, that fell into each protein category.

wheats grown over a wide range of territory exhibiting a large range of soil types and climatic conditions. Even within a single wheat-producing area, as, for example, in the spring wheat area of the United States, there may be a large variability in certain seasons, and a greater variability when the production of a succession of seasons is pooled.

In König's³ extensive tabulation of wheat composition, winter wheats grown in southern and western Germany are reported to have contained

³ "Chemie der menschlichen Nahrungs- und Genussmittel," Band I, S. 431-483, 3rd Aufl., Berlin, 1889.

from 8.83 to 19.01 per cent of protein (recorded as "nitrogenous-substance," and without the protein conversion factor), with an average of 12.29 per cent. Spring wheats from the same area exhibited an even wider range, and averaged 14.95 per cent. When all the German wheats are compared, the extremes include a minimum percentage of 7.60, a maximum of 21.18. The lowest percentage of protein reported by König was 7.07 in a wheat grown in Scotland, the highest in a wheat from the Russian Caucasus, 24.16. Both of these are from early studies, published in 1865 and 1854 respectively, before the Kjeldahl method was known, and hence may be open to some doubt as to accuracy. Even though there is some error attached to these determinations, it is apparent that the protein range in wheats of the world is very high, and espe-

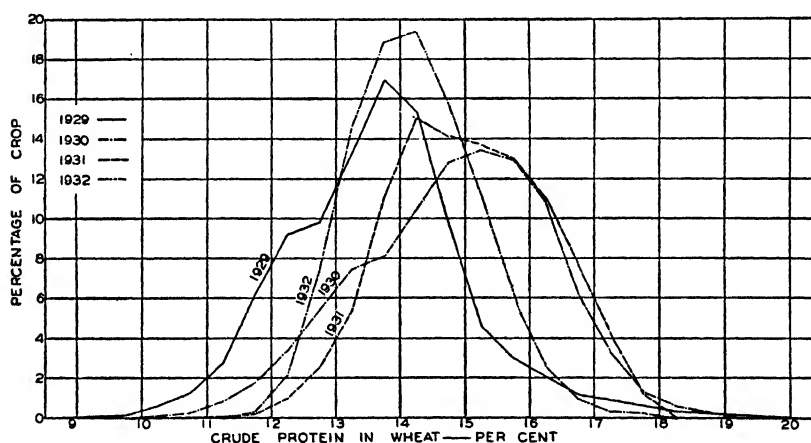


FIG. 3. Distribution curves showing the percentages of all hard spring wheat, crops of 1929, 1930, 1931, and 1932 marketed through Minneapolis, Minnesota, that fell into each protein category.

cially since recent and more acceptable analyses of wheats in the United States have revealed nearly as wide a range as these earlier data covering the entire world. Teller ⁴ reported 8.04 per cent of crude protein ($N \times 5.7$) in a sample labeled "White wheat, Canada," and 19.15 per cent in "Red wheat, South Dakota." White Australian wheat grown in California,⁵ 1910 crop, contained 6.92 per cent of crude protein. Numerous California wheats were reported as containing less than 8.5 per cent crude protein. As against these lower values, the author ⁶ found that over 25 per cent of the spring wheat marketed through Minneapolis in the crop season of 1936

⁴ Ark. Agr. Exp. Sta. Bull. 42 (1896).

⁵ Calif. Agr. Exp. Sta. Bull. 212 (1911).

⁶ Minn. Agr. Exp. Sta. Tech. Bull. 147 (1941).

contained more than 17.0 per cent of crude protein; 120 carloads of wheat averaged more than 19 per cent crude protein.

These extremes, while of interest, are of less significance than the means, the distribution, and the consequent standard deviations of the protein content of wheat crops grown over a large producing area. Protein data for the spring wheat crops from 1925 to 1938 were thus organized by the author⁶ and the distribution curves for these samples, totaling over 430,000 separate analyses, are presented in Figures 2, 3 and 4.

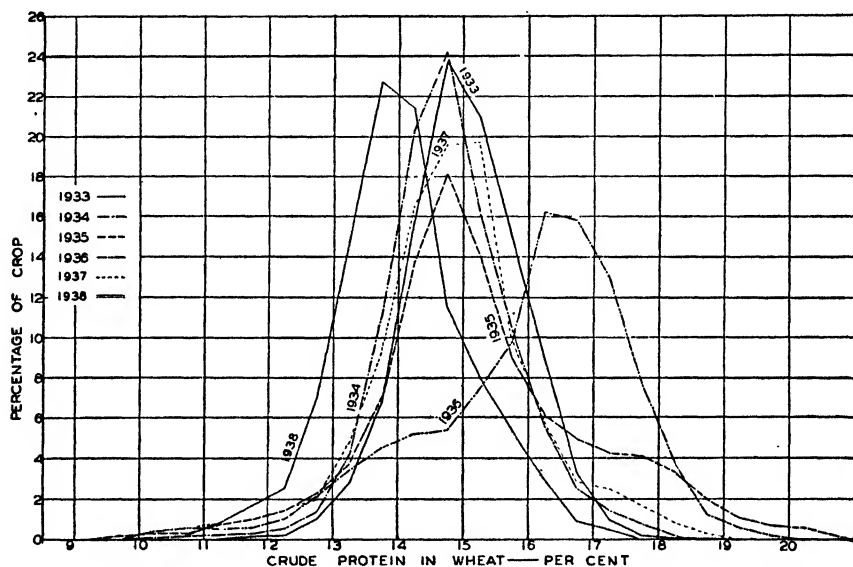


FIG. 4. Distribution curves showing the percentages of all hard spring wheat, crops of 1933, 1934, 1935, 1936, 1937, and 1938 marketed through Minneapolis, Minnesota, that fell into each protein category.

Without indulging in an extensive analysis of these data here, or the reasons for the large differences between certain of the crop seasons, it is apparent from these graphs that the wheat crops of 1925, 1927 and 1928 were lower in protein content than the succeeding crops, and in two of these seasons, 1927 and 1928, the variability in terms of protein content was relatively small. These were seasons of normal rainfall and general climatic conditions. As the drouth conditions in the northern Great Plains Area became more and more acute during the seasons from 1929 to 1936, there was a general advance in protein content, and a trend toward greater variability in this characteristic of the wheat crop.

The more significant data respecting these 14 spring wheat crops are recorded in Table 42, and those interested are referred to the author's

Table 42. Average Percentages of Protein in Spring Wheat Marketed Through Minneapolis, Minnesota, by Crops.

Crop Year	Minnesota			North Dakota			South Dakota			Montana			All spring wheat—		
	No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)	Standard deviation σ
1925	6,609	11.90		17,204	11.22		3,694	12.47		5,739	14.30		33,246	12.49	1.34
1926	7,051	12.47		11,716	13.19		1,886	14.02		5,492	14.26		26,145	13.28	1.55
1927	6,075	11.70		31,323	11.82		13,365	12.26		13,181	12.14		63,944	11.96	0.78
1928	4,280	12.46		24,974	12.23		6,745	12.69		13,965	12.60		49,964	12.42	0.77
1929	4,737	12.05		19,400	13.75		8,422	13.64		4,643	13.30		37,202	13.70	1.41
1930	6,710	12.95		29,532	14.75		9,938	15.50		5,869	15.80		52,041	14.85	1.47
1931	4,370	14.00		9,939	15.20		2,776	15.66		97	16.88		17,182	15.00	1.22
1932	5,237	13.87		24,802	14.11		12,661	14.12		2,327	15.10		45,027	14.21	0.99
1933		23,829	15.03	0.89
1934		12,900	14.80	1.04
1935		28,544	15.30	1.71
1936		16,698	15.92	1.64
1937		12,185	14.83	1.28
1938		13,169	18.78	1.04
															11.5

Table 43. Average Percentages of Protein in Soft Red Winter Wheat.

Crop harvested in	Ohio			Illinois			Indiana			Michigan			All red winter wheat—		
	No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)		No. of samples	Ave. protein (%)	Standard deviation σ
1931	1968	10.31		3	9.75		994	10.19		96	10.18		3061	10.26	0.67
1932	1934	9.82			1342	9.85		153	9.52		3429	9.82	0.49
1933	2357	10.63			1296	10.73		145	10.38		3798	10.65	0.69
1934	2527	11.69			2658	11.53		125	11.22		5310	11.61	0.82
1935	3709	9.72			1479	10.33		144	9.81		5332	9.89	0.64
1936	2903	9.42		178	10.04		1551	9.56			4632	9.49	0.49
1937	1789	9.35		396	9.86		1765	9.24		73	8.85		4501*	9.35*	0.59
1937		Kentucky 478	9.44	

* Including the Kentucky samples.

more extended paper on this subject ⁶ for further details. Somewhat similar data for the soft red winter and white winter wheat crops from 1931-1937 inclusive, as grown in the Central States, are recorded in Tables 43 and 44, and are presented graphically in Figures 5 and 6. It is evident that the average protein content of these soft winter wheats is lower than

Table 44. Average Percentages of Protein in White Winter Wheat.

Crop	Ohio		Indiana		Michigan		All white winter wheat		
	No. of samples	Ave. protein (%)	No. of samples	Ave. protein (%)	No. of samples	Ave. protein (%)	No. of samples	Ave. protein (%)	Standard deviation σ
1931	50	9.70	2	9.75	300	10.54	352	10.30	0.81
1932	95	9.09	402	9.00	497	9.01	0.41
1933	64	10.58	243	9.84	307	9.92	0.58
1934	84	10.95	161	11.32	245	11.19	0.67
1935	175	8.83	402	9.03	577	8.97	0.54
1936	241	8.98	284	8.98	525	8.98	0.43

that of the hard spring wheats, and, moreover, their relative variability in this characteristic was lower, as evidenced by the standard deviation recorded in the table, and the general spread of the curves. Similar analyses of wheat crops have been reported from the Canadian Grain Commission Laboratory in Winnipeg, but aside from a few sources, world data are not conveniently available in adequate completeness. An organization and similar presentation of protein, or gluten data from other areas in America, and other major wheat-growing regions of the world would be very useful.

Nearly 4,000 analyses of Rumanian wheats of the six crops from 1900-1905 inclusive, previously published by Zaharia ⁷ were subjected to statistical analysis by Berczeller and Wastl.⁸ Certain of the constants computed by them are not familiar to American biometricians, and must be explained briefly. Under the term "Variationsbreite" they describe the range of the distribution by dividing the highest category by the lowest. Thus, for protein content the highest category or class, when the samples were grouped into class with a one per cent range, had the median value of 21.5 per cent. The lowest category had a median value of 8.5 per cent. Then $21.5/8.5 = 2.53$, which is the "Variationsbreite" for protein content. Like constants were calculated for three other constituents as follows: fat, 1.83; cellulose, 2.03; and ash, 2.18. Thus the sequence in terms of this crude measure of variability becomes:

Protein > ash > cellulose > fat.

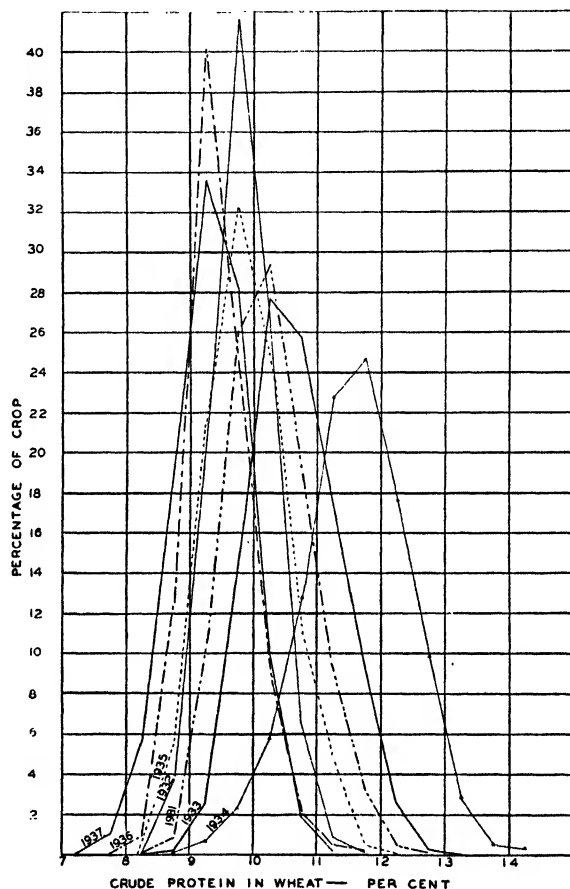
This is not regarded as a very acceptable measure of variability,

⁷ "Le blé Roumain," 581 pp., Bucharest, 1910.

⁸ *Biochem. Z.*, 177, 168 (1926).

however, since it tends to attach too much significance to stray, freak samples which fall outside of the principal population. In several instances the class which determines the entry into the equation included only one sample. A superior computation was supplied by Immer⁹ in the form of

FIG. 5. Distribution curves showing the percentages of Central States soft red winter wheats, crops of 1932 to 1938 inclusive, that fell into each protein category.



the standard deviation (σ), and this, in turn, was converted into the coefficient of variation (C.V.) by dividing σ by the mean. These constants, together with the means, are as follows:

	Crude Protein ($N \times 6.25$)	Fat	Cellulose	Ash
Mean, per cent	13.55	1.85	2.56	2.02
Standard deviation	1.99	0.14	0.25	0.21
Coefficient of variation (%)	14.7	7.6	9.8	10.4

⁹ Private communication, July, 1941.

The coefficients of variation fall in the same order as Berczeller and Wastl's "Variationsbreite" values, but the actual mathematical C. V. values are more significant. Thus it appears that the actual relative variability of protein content of these wheats was about double that of the fat content, and about half again as great as the ash and cellulose content.

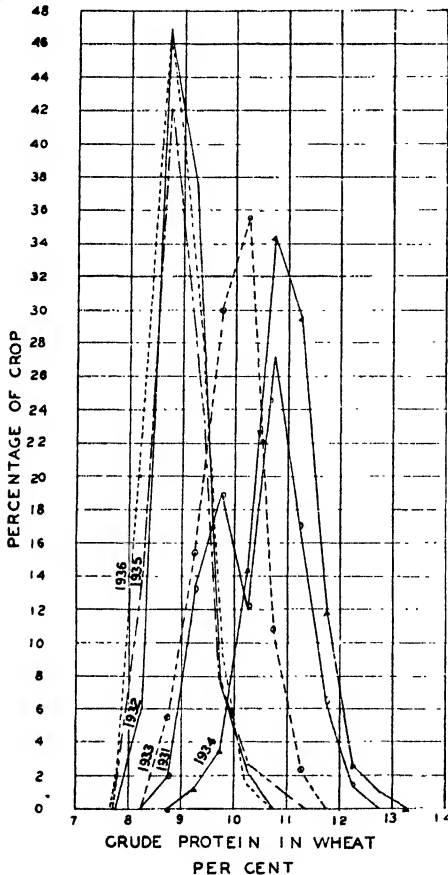


FIG. 6. Distribution curves showing the percentages of Central States white winter wheats, crops of 1932 to 1937 inclusive, that fell into each protein category.

The means of these analyses are also significant for themselves alone and particularly in the instance of the protein content. Presumably all of them are presented on the dry-matter basis. Thus, on the 13.5 per cent moisture basis, so commonly used in America, and use of the factor $N \times 5.7$ instead of $N \times 6.25$ as used by Zaharia, these wheats contained an average of 10.72 per cent of crude protein. This is about the same range of protein content as American semi-hard winter wheats, and some-

what above the general run of average soft red winter wheats grown in the Central United States.

Not only have large variations been observed in the average protein content of wheat grown in different plots or fields, but also among the kernels produced by a single plant, as shown by Gericke.¹⁰ He planted wheat in soil deficient in nitrogen so as to restrict stalk formation to one culm per plant during the early growth period. Ninety days after planting, NaNO_3 was applied and this induced tillering. Thus he secured two crops of stalks on a plant, and since the supply of nitrogen provided by the added nitrate was more than the parent stalk could absorb, but less than the new tillers could utilize, he fulfilled the desired conditions, which resulted in producing high-protein grain in the later growth period on some stalks of the plant, and low-protein grain on other stalks. The result was a large variation in protein content in the grain produced by stalks of the same wheat plant, as shown by Gericke's data recorded in Table 45. It is problematical whether or not equivalent variations would appear under natural field conditions, but it is significant that the wheat plant can respond to its chemical environment in this manner.

Table 45. Variation in the Percentage of Protein in the Grain of Different Stalks of the Same Wheat Plant, as Obtained from the Chemical Manipulation of the Soil by Gericke.¹⁰

Variety	Percentage of protein		Difference	
	Low	High	Actual	Percentage
Bunyip	13.6	17.6	4.0	30
Cedar	12.3	18.6	6.3	57
Dart's Imperial	10.9	11.1	0.2	2
Early Baart	10.4	12.2	1.8	17
Fulcaster	8.2	11.4	3.2	39
Hard Federation	11.8	17.3	6.5	55
Sonora	6.4	14.0	7.6	119
White Australian	10.2	13.0	2.8	27

This matter of the variation of protein content of kernels produced by wheat plants was explored further by Knyaginichev,¹¹ who subjected individual grains to micro-analysis. In an individual ear the middle spikelets produced kernels of higher average protein content than the upper and lower spikelets. Within individual spikelets the heavier grains contained more protein, both in absolute amounts and in percentage. The correlation between these two characters ranged between $r \pm m = 0.58$ to 0.72 . Further confirmation of this conclusion was afforded by an extension of these studies by the same investigator.¹²

In a study of the amino-acids in the protein of the entire wheat kernel,

¹⁰ *Science*, 71, 73 (1930).

¹¹ *Bull. Applied Botany, Genetics, Plant Breeding (U.S.S.R.)*, ser. 3, 15, 5 (1936).

¹² *Ibid.*, 15, 55 (1936).

with HCl as a hydrolyzing agent, and then determining cystine by Sullivan's method, tryptophane by May and Rose's method, tyrosine by the method of Folin and Ciocalten, as well as the basic amino acids, the results recorded in Table 46 were obtained by Csonka.¹³ Note that the values

Table 46. Amino-Acid Content and Total Nitrogen in Percentages of Moisture-free Wheat, as Reported by Csonka.¹³

Fraction	Variety and place grown		
	Marquis-hard spring North Dakota	Tenmarq-hard winter Kansas	Fulhio, soft winter Ohio
Cystine	0.270	0.170	0.157
Tryptophane	0.18	0.13	0.16
Tyrosine	0.866	0.560	0.405
Arginine	0.510	0.376	0.356
Histidine	0.280	0.117	0.080
Lysine	1.510	1.225	0.872
Total nitrogen	3.31	2.58	2.34

for tryptophane as published in his paper have been doubled, this correction having been supplied by the author. In his opinion the protein quality, from the nutritive standpoint, is better in wheat varieties of higher nitrogen content than in those of low nitrogen. The nutritive value of the protein of whole-wheat flour was regarded as comparing favorably with that of casein, although tryptophane is evidently at a lower level, as shown by the calculations given in Table 47.

Table 47. Protein Quality as Shown by Relationship between Amino-Acids and Total Nitrogen Content, as Reported by Csonka.¹³

	Mg of indicated amino acid per gram of total nitrogen					
	Cystine	Tryptophane	Tyrosine	Arginine	Histidine	Lysine
Marquis	81	56	262	154	85	456
Tenmarq	65	52	173	146	43	475
Fulhio	67	68	217	152	34	373
Casein for comparison	20	130	405	236	156	475

During the maturation of the wheat kernel, the Hausmann fractions separated from the acid hydrolyzate of the grain were shown by Thatcher¹⁴ to undergo a progressive change. In a sample of bluestem spring wheat, between the 8th day following blossoming, and complete development, the change in the several fractions was as follows: Amid nitrogen, 12.5 to 17.2 per cent; humin nitrogen, 13.5 to 6.8 per cent; basic nitrogen, 21.0 to 16.3 per cent; mono amino nitrogen, 54.3 to 61.4 per cent. This suggests that the types of nitrogenous substances in the kernel during the development period were not constant in kind or proportion, but the data scarcely permit of a more adequate analysis of these relationships.

¹³ *J. Biol. Chem.*, **118**, 147 (1937).

¹⁴ *J. Am. Soc. Agron.*, **7**, 273 (1915).

In the range between 0.1*N* and 2.0*N*, Mangels and Martin¹⁵ found that the lowest concentration of lactic, tartaric, citric and oxalic acids dispersed the most protein of five hard spring wheat flours. Acetic acid was an exception, although the difference effected by 0.1*N* and 0.5*N* was small, and a further increase in concentration resulted in a slight decrease in dispersion of the protein. In general these five organic acids effected the dispersion of most of the flour protein, 0.1*N* oxalic acid dispersing the least, *viz.*, 75 per cent, while the other four dispersed about 85 per cent. Propionic, butyric, valeric and succinic acids at 0.1*N* concentration effected about the same dispersion as oxalic, in the range of 72-76 per cent. Phosphoric acid dispersed much more protein in the range of normality here involved, *i.e.*, from 0.1*N*-2.0*N*, than did HCl or H₂SO₄. The last two were moderately effective at 0.1*N*, dispersing 40-45 per cent of the protein, but when a concentration of 0.5*N* was reached or exceeded, the dispersed protein represented only about 20 per cent of the total that was present.

The binding of glacial phosphoric acid by flour proteins was measured by Samuel and Schofield¹⁶ by shaking them up with an excess of the acid and determining the amount remaining in solution by back-filtration of an aliquot. In the instance of 37 flours that were examined, the capacity to bind glacial phosphoric acid ranged from a minimum of 6.7 to a maximum of 10.1 milliequivalents per 100 g of flour. While the high protein flours in general had the highest uptake, nevertheless the acid bound per gram of nitrogen varied between 3.52 and 4.82.

If the lowest pH at which the proteins could be washed free from ions was accepted as the isoelectric point, then this point for gluten was pH 6.1, for gliadin pH 6.85, and for glutenin 5.5.

Plant proteins could be differentiated by the precipitin reaction through the use of specific sera, according to Gasis.¹⁷ Cereals, legumes and other plant types were included in the study, and it appeared that the reaction was weaker or more pronounced according to whether the phylogenetic relation is remote or close. In Gasis' experience plant proteins could be differentiated more readily by the precipitin reaction than animal proteins.

Zein, the alcohol-soluble protein of corn or maize, did not cause an anaphylaxis reaction in animals sensitized with gliadin of wheat, nor with hordein of barley in the animal experiments conducted by Wells and Osborne.¹⁸ Later,¹⁹ they reported that guinea pigs sensitized with wheat gliadin or rye gliadin gave strong anaphylactic reactions with hordein, but

¹⁵ *Cereal Chem.*, **12**, 149 (1935).

¹⁶ *Trans. Faraday Soc.*, **32**, 760 (1936).

¹⁷ *Berlin klin. Wochschr.*, **45**, 858 (1908).

¹⁸ *J. Infectious Diseases*, **8**, 66 (1911).

¹⁹ *Ibid.*, **12**, 341 (1913).

these reactions were not as strong as those obtained with the homologous protein (*i.e.*, wheat gliadin interacted with rye gliadin). They accordingly concluded that the specificity of this reaction is determined by the chemical constitution of the protein rather than by its biological origin.

Gliadin and glutenin also reacted anaphylactically with each other, and hence were assumed to contain common reactive groups. They suggest that the entire protein molecule is not involved in the specific character of the anaphylaxis reaction, but that the reaction is developed by certain groups contained therein; moreover, that one and the same-protein molecule may contain two or more such groups.

Rabbits immunized with wheat gliadin by Lake, Osborne and Wells²⁰ yielded a serum which gave a specific complement fixation reaction with wheat gliadin in dilutions as high as 1-100,000, but not with rye gliadin or barley hordein in dilutions of 1-2,000. These sera failed to give positive precipitin reactions with wheat gliadin in 1-2,000 dilution, the most concentrated solution of gliadin that could be used.

On the other hand, rabbits immunized to hordein (2.63 g each) yielded an antiserum of high titer, as shown by a complement fixation reaction in dilutions of 1-400,000 to 1-600,000. It reacted only to hordein, however, and failed to react to gliadin from wheat or rye.

Rabbits immunized with about twice as much hordein (5.6 g each) yielded a different antiserum, which gave the complement fixation reaction not only with hordein in high dilution, but also with gliadin from wheat and rye. The precipitin test gave some degree of quantitative specificity, the reaction being given in the highest dilution with hordein, in lower dilution with gliadin (*i.e.*, with a more concentrated solution), and in lowest dilution with rye gliadin.

When Wodehouse²¹ began his immunochemical studies of the wheat proteins he expected to find that some one of them was entirely responsible for the anaphylactogenic properties of that cereal, or else that they all behaved in the same fashion. On the contrary, his investigations showed that, though all the proteins were capable of effecting anaphylactic symptoms, their method of action is complex and not capable of simple analysis. While all were anaphylactogenic, no two behaved exactly alike. Heavy cooking of the cereals until they acquired a light tan color resulted in a loss of this activity.

Crude Gluten

In mill control, as well as in merchandising wheat and flour, it has been the common practice in the United States for the past score of years to

²⁰ *Ibid.*, 14, 364 (1914).

²¹ *Am. J. Botany*, 4, 417 (1917).

determine and report the crude protein content of these materials, rather than the content of "crude gluten." Some American laboratories have been engaged in recovering crude gluten from flour by washing it from the dough, however, thus affording an opportunity to observe the physical properties of this protein complex. Crude gluten determinations are not regarded as quantitative procedures on this side of the Atlantic as a general rule; and even where the gluten is prepared for observation, protein content is commonly determined by chemical methods in the interest of accuracy. In Europe the preference is for the crude gluten determination, however, and this practice is of long standing, as evidenced by the detailed description of the gluten washing process in 1856 by Rivot.²² Several ingenious gluten-washing devices have been designed and used to reduce the hand labor involved in recovering this material from a flour dough. Also a number of methods have been proposed for measuring certain physical properties of the wet crude gluten thus recovered, and these have been described elsewhere by the author.²³ Also numerous procedures involving the determination of the viscosity of flour suspensions, in which acidulation or some other manipulation was employed to strongly hydrate the gluten proteins, have been proposed. It is beyond the scope of this book to describe these methods, or to discuss the interpretation of the measurements. It would require a large chapter to cover this phase of the subject, and no general agreement can be reached from the data presented or the conclusions drawn by various investigators. Suffice it to say that, in numerous of the studies with hard wheat, the correlation of viscosity data with the results of baking tests is scarcely greater than that of crude protein determinations with the same baking tests.

Dry crude gluten, as recovered by washing dough in water, and drying it to constant weight, is not comprised solely of proteins. The presence of fat in gluten from fine flour was noted by von Bibra²⁴ to the extent of about 6 per cent, and Ritthausen²⁵ found both fat and starch in gluten. Wanklyn and Cooper²⁶ reported 1 per cent fat, and 0.3 per cent ash (on the flour basis) in dry gluten, which would be equivalent to about 10 per cent fat and 3 per cent ash in the dry gluten itself. Osborne and Voorhees²⁷ found in the gluten residue remaining after the extraction of gliadin with alcohol "a considerable amount of phytocholesterin and lecithin together with fat" that could be washed out with ether. Indeed, the proportion of non-protein substances present may represent about one-fifth

²² *Ann. chim. phys.*, 3rd ser., 47, 50 (1856).

²³ *Wheat Studies of the Food Research Institute*, 16, No. 6 (1940).

²⁴ "Die Getreidearten und das Brod," Nürnberg, 1860.

²⁵ *J. prakt. Chem.*, 91, 296 (1864).

²⁶ "Bread Analysis. A Practical Treatise on the Examination of Flour and Bread," 1881.

²⁷ *Am. Chem. J.*, 15, 392 (1893).

of the total substance, according to Norton's ²⁸ observations. He reported the composition of a typical dry crude gluten as follows:

Ether extract	4.20%
Carbohydrates (not including fiber)	9.44%
Fiber	2.02%
Ash	2.48%
Gliadin	39.09%
Glutenin	35.07%
Soluble protein (NaCl-sol.)	6.75%
	<hr/> 99.05%

The crude glutens examined by Olson ²⁹ were somewhat variable in composition, as shown by the range in nitrogen content. Thus in 24 samples the maximum nitrogen content was 14.09 per cent, the minimum 11.28 per cent, with an average of 12.77 per cent. Using the factor $N \times 5.7$, this average would represent about 73 per cent of protein in the dry crude gluten. Moreover, Olson apparently did not recover all the nitrogen of the flour in the form of nitrogen in the crude gluten; in fact, the average recovery was about 73 per cent. Based upon current assumptions respecting the ratio of gluten proteins to total protein, this represents a net loss of 10 per cent or more of the gluten proteins. This loss of gluten proteins is even greater if Norton's ²⁸ data are correct, since he showed the presence of non-gluten or "soluble" proteins in his analysis of crude gluten.

Olson also demonstrated quantitatively what had been observed previously in a general way, namely that in low-protein flours it may become difficult to recover all the crude gluten by direct washing of the dough with water. Losses of 2 to 4 per cent of protein (based on the flour) were experienced in recovering gluten from flours containing 8 per cent or less of total protein. In fact, it has been observed by the author that with some low-protein flours, particularly clear or low grades milled from low protein wheats, gluten may fail to agglutinate at all, and be washed away in the stream of water used in washing the starch and soluble material out of the dough. In a practical way, this difficulty has been corrected at times by mixing such flours with highly refined high protein flour, and then correcting the results by subtracting the contribution of gluten made by the latter.

The analysis of dry gluten reported by Gerum and Metzger ³⁰ showed the presence of 0.26 per cent ether extract, 0.58 per cent crude fiber, 0.346 per cent P_2O_5 and 89.4 per cent protein ($N \times 5.7$).

An extensive study of the composition of crude gluten in relation to the composition of the flour from which it is derived was reported by Dill. ³¹

²⁸ *J. Am. Chem. Soc.*, 28, 8 (1906).

²⁹ *J. Ind. Eng. Chem.*, 4, 206 (1912).

³⁰ *Z. Nahr. Genussm.*, 46, 74 (1923).

³¹ *Cereal Chem.*, 2, 1 (1925).

In the instance of gluten recovered from eastern soft red winter wheat, the composition shown in Table 48 was recorded.

Table 48. Composition of Dry Crude Gluten as Reported by Dill.³¹

	In crude gluten (%)
Lipoids (neutral extraction method)	7.05
Ash	0.63
Carbohydrates (acid hydrolysis)	18.82
Protein (N×5.7)	72.67*
	<hr/> 99.17
Additional detailed data:	
Carbohydrates (Pflüger glycogen method)	17.52
Total P ₂ O ₅	0.38
Phytin, P ₂ O ₅	0.05
Lipoid, P ₂ O ₅	0.10
Inorganic, P ₂ O ₅ (by difference)	0.23
Calculated phosphatide (as distearyl lecithin)	1.13
Lipoids (acid digestion method)	5.01

* Represents 84.5% of the protein of the original flour.

Crude gluten was found by Gerum and Metzger³² to contain about one-fifth of the total phosphorus of the flour from which it was recovered.

Crude gluten from durum wheat was observed by Mangels³³ invariably to contain more ash and phosphorus than common or bread wheat glutes. Also the fatty material from the two sources had different physical and chemical constants.

Various investigators demonstrated the effect of several factors upon the quantity and physical properties of wet crude gluten recovered from the same lot of flour. Arpin³⁴ observed the yield of gluten to increase when the temperature of the water used in its recovery was increased from 5 to 25° C. The length of time that the dough stood before the gluten was washed from it, length of the washing period, and mechanical manipulation in the washing operation all exerted an influence upon the yield of gluten, in the experience of Kepner.³⁵ The necessity of a "rest period" of standing in the dough stage before washing out the gluten in order to obtain an adequate yield of gluten had been observed much earlier by Benard and Girardin.³⁶

One of the most prominent variables in its effect upon gluten properties is the composition of the water supply used in making and washing the doughs. As early as 1883 Balland³⁷ was aware that the use of hard water would increase the yield of gluten over that obtained with distilled water.

³² *Z. Untersuch. Nahr. u. Genussm.*, 44, 86 (1922).

³³ *No. Dak. Agr. Exp. Sta. Bull.* 253, p. 55 (1930).

³⁴ *Ann. chim. anal. chim. appl.*, 7, 325 (1902).

³⁵ *J. Ind. Eng. Chem.*, 6, 481 (1914).

³⁶ *J. pharm. chim. 5th ser.*, 4, 127 (1881).

³⁷ *J. pharm. chim.*, 8, 443 (1883).

One of the classic pieces of work in the field of colloid chemistry was the observation by Wood and Hardy³⁸ on the effect of electrolytes on the physical state of gluten. They found that gluten washed out of flour with distilled water was lacking in ductility and tenacity; that acids and alkalis effect a dispersion of gluten as a function of their concentration; and that salts lessen the power of acids and alkalis to destroy the cohesion of gluten. It appears safe to suppose that these studies directly or indirectly stimulated more research on gluten properties in the two decades following their publication than any other single contribution since the earlier papers of Osborne and co-workers. It led to the suggestion by Jacobs,³⁹ LeClerc,⁴⁰ and Dill and Alsberg,⁴¹ that a synthetic "hard" water, made by dissolving definite quantities of salts in distilled water, be employed in lieu of tap water in washing out the crude gluten. In fact, Dill and Alsberg's saline solution was made with sodium phosphates adjusted to pH 6.8.

A 2 per cent NaCl solution adjusted to pH 6.8 with a phosphate mixture was advised by Berliner and Koopman⁴² for the separation of crude gluten from dough by a washing process. No standing of the dough before washing was found necessary. Moreover, the practice advised made it possible to recover glutens from low-grade flours, from which no gluten could be obtained by washing with tap water. Berliner and Koopman further concluded that gluten is formed in the kernel during the ripening of the wheat and is not formed in the dough.

The use of the saline solution recommended by Berliner and Koopman in washing crude gluten from dough was approved by Marotta and Vercillo.⁴³ They observed, moreover, that if the resulting crude gluten was dried *in vacuo* at 80° its properties are maintained, including the solubility of certain portions of it in 70 per cent ethanol.

Even when such a solution was used, it appeared from the collaborative studies conducted by Kent-Jones and Herd⁴⁴ that this did not eliminate the principal errors of the determination of crude gluten. Personal differences in manipulation introduced substantial variations in the results obtained by nine collaborators, although each operator's results were fairly consistent, as evidenced by the ratio between the nitrogen of the flour and the percentage of dry gluten.

A study of the gluten washing process was made by Fisher and Halton,⁴⁵ who found that the principal factors affecting the accuracy of

³⁸ *Proc. Roy. Soc. London B*, 81, 38 (1908).

³⁹ Mimeographed report to Amer. Soc. Mfg. & Baking Tech., 1915.

⁴⁰ *J. Assoc. Official Agr. Chem.*, 4, 180 (1920).

⁴¹ *Cereal Chem.*, 1, 222 (1924).

⁴² *Z. ges. Mühlenw.*, 6, 84 (1929).

⁴³ *Ann. chim. applicata*, 22, 777 (1932).

⁴⁴ *Analyst*, 52, 439 (1927).

⁴⁵ *Cereal Chem.*, 13, 575 (1936).

gluten determinations are: (1) the quantity of flour used; (2) the composition of water used in washing; (3) temperature of the water; (4) treatment of the dough prior to washing, with particular reference to the duration of the dough mixing and the rest period; (5) time interval elapsing during the kneading or washing process; and (6) personal factors varying with the operator. Since the last-named appeared to be very prominent, they recommended the use of a mechanical gluten-washing apparatus. When tap water of sufficient hardness is not available they concluded that 0.1 per cent NaCl solution should be used in washing glutes.

The use of dry crude gluten to supplement low-gluten flour doughs in baking was attempted by Harris⁴⁶ with fairly favorable results in terms of bread quality when the dry gluten was recovered from suitable hard wheats. A gluten preparation from soft winter wheat flour was negligible in effect, and a durum wheat flour gluten decreased the loaf volume in test bakes. In the 14 specimens of dried gluten prepared in these studies there was an average of 1.09 per cent ash, and 65.8 per cent crude protein, on a 13.5 per cent moisture basis. This would represent about 75 per cent of crude protein and 1.25 per cent of ash on a dry basis. The other constituents of the crude gluten were not reported.

The purity of Harris' glutes appears to have been somewhat lower than those prepared by Smirnov,⁴⁷ who reported that his preparations contained from 84 to 88.4 per cent of pure gluten. When the washing with water was prolonged, the purity was elevated to 92 per cent. Ash and fat content of the gluten increased as the flour quality decreased.

Gluten from Italian wheat was hydrolyzed by Padoa,⁴⁸ apparently without first fractionating the various proteins in the mixture. In the hydrolyzate he identified the following amino-acids in the percentages given: aspartic acid 9.60; leucine 8.60; phenylalanine 4.08; alanine 5.02; glycine 8.62; proline 8.05; glutamic acid 26.78; tyrosine 1.34; histidine 0.97; arginine 1.89; tryptophane 1.24; lysine 1.23; cystine 1.43; and valine 3.27. In his judgment, certain amino-acids of greatest physiological importance, such as lysine, tryptophane and cystine, are present in considerable proportions.

Dispersed gluten was hydrolyzed somewhat by 0.1N NaOH and 0.1N CH_3COOH in the experiments conducted by Cook and Rose,⁴⁹ as indicated by the percentage of the protein which was not precipitated by tannic acid, and by trichloroacetic acid. The hydrolyzing action of the acid and the alkali was accelerated by elevating the temperature to 80° C, particularly

⁴⁶ *Cereal Chem.*, 17, 222 (1940).

⁴⁷ Mukomol's, 13, 34 (1938); *Chimie & industrie*, 41, 764; *Chem. Abs.*, 33, 5919. Original not seen.

⁴⁸ *Ann. chim. applicata*, 21, 544 (1937); *Chem. Abs.*, 32, 6297 (1938). Original not seen.

⁴⁹ *Can. J. Research*, 12, 248 (1935).

in the instance of the alkaline reagent. No appreciable hydrolysis occurred when the gluten was dispersed in 8 per cent sodium salicylate solution, or in 30 per cent urea solution.

While the researches of T. B. Wood and W. B. Hardy, and the later researches of others which they inspired, did much to account for certain peculiar and variable properties of the gluten proteins, the question as to why they formed the loose combination found in wet crude gluten was not wholly solved by their approach. The earlier suggestion by Weyl and Bischoff,⁵⁰ to which reference has been made, that gluten was formed in consequence of enzyme action which effected a coagulation analogous to the enzymic theory of blood coagulation did not receive much support.

In the polemic relative to the pre-existence of gluten in wheat, Balland⁵¹ asserted unequivocally that it did pre-exist. Apparently his decision was based in large part upon the observation that it could be recovered from an ice-cold (2° C) dough, as well as from a dough treated with a disinfecting H₂SO₄ solution (in the presence of salt). These treatments were presumed to inhibit any fermentations that might result in gluten formation from simple, pre-existent substances.

Whether gluten was formed by enzymes or was a hydrated complex formed by the reaction of gliadin and glutenin could not be determined by Gershzon⁵² from the experimental data available to him.

Obviously there was something lacking in these earlier hypotheses, which appears to have been supplied recently, at least in part, by the development of the concept of coacervation. In its simplest terms, coacervation may be described as a special form of mutual precipitation. Individual micelles or aggregates bearing different charges (sometimes described as "opposite" charges, although this is a relative term which must be interpreted in terms of some more or less arbitrary reference base) which retain their identity, but are held together in a coagulum or swarm by electrostatic attraction, constitute a coacervate. While the phenomenon had been known and described long before, the brilliant exploitation of this principle by Kruyt, H. G. Bungenberg de Jong, and associates did much to focus attention upon it as a possible means for explaining various biological and other phenomena. Numerous of their papers in *Kolloid-Zeitschrift*, *Kolloid Beihefte* and *Biochemische Zeitschrift* during the decade beginning in 1929 contain the results of their observations. A convenient summary by H. G. Bungenberg de Jong appeared in "La coacervation, les coacervats, et leur importance en biologie."⁵³

Since the electrostatic charge on a micelle is affected by its environ-

⁵⁰ *Ber.*, 13, 367 (1890).

⁵¹ *Compt. rend.*, 116, 202 (1893).

⁵² *Colloid J. (U.S.S.R.)*, 3, 915 (1937); *Chem. Abs.*, 32, 5869 (1938). Original not seen.

⁵³ "Actualités scientifiques et industrielles," Nos. 397 and 398, Hermann et Cie, Paris, 1936.

ment, it follows that the association of micelles may be disturbed or altered by charges in the composition of the medium with which they are in contact. This accounts in part for the profound effects of hydrogen- and hydroxyl-ions, complicated further by other ions of different valence, and in varying concentration contributed by salts. The normal sequence of events in such a complex may be interfered with by the further addition or presence of phosphatides and lipids, possessing a tendency to orient on hydrated surfaces.

In an extensive series of papers dealing with the colloid chemistry of gluten, H. L. Bungenberg de Jong and W. J. Klaar⁵⁴ lay a basis for the development of a hypothesis which involves properties essentially similar to those included in the coacervation concept. Their researches included extensive use of viscosity and turbidity measurements as convenient, though indirect, criteria of the hydration and coagulation occurring in consequence of the manipulations to which they subjected gluten proteins, notably gliadin. A convenient consolidation of certain significant observations on the individual gluten proteins was also presented by them,⁵⁵ and later⁵⁶ they discussed the properties of mixtures of gliadin and glutenin. This work was summarized and discussed by de Jong⁵⁷ in his paper on gluten formations.

de Jong pointed out that when viscosity or turbidity was used as a criterion, the results of mixing varying ratios of gliadin and glutenin are additive, except in a specific pH range. Moreover, it was clear that the range of deviation from the rule of additive behavior was limited by the pH values at which the two components are isoelectric. This deviation from the additivity law may be interpreted to imply an interaction between the components under observation. This interaction must be caused by a difference in the electrical charge upon the particles of the two components. In the region between the two isoelectric points the gliadin is positively charged, the glutenin negatively charged. At all other pH values the two components possess the same sign of charge, either both negative or both positive. In de Jong's opinion, different methods of investigation all point to the correctness of this assumption.

When the sign and magnitude of the charge on gliadin and glutenin particles are progressively changed they must pass through a certain pH value at which the total charge of the positive component (gliadin) will be compensated by the total charge of the negative component (glutenin). This will represent the point of maximum interaction and complex forma-

⁵⁴ *Cereal Chem.*, **6**, 373 (1929); **7**, 222 (1930); **7**, 587 (1930); **8**, 439 (1931).

⁵⁵ *Trans. Faraday Soc.*, **28**, 27 (1932).

⁵⁶ *Trans. Faraday Soc.*, **28**, 798 (1932).

⁵⁷ *J. Soc. Chem. Ind.*, **52**, 391T (1933).

tion. This point can be identified as the maximum of turbidity, for example. In a diagrammatic representation of this sequence, de Jong presents a curve in which the graph of ascending turbidity passes through a fairly sharp maximum in the range of pH 6.1, and recedes substantially on either side of this maximum in approaching pH 6.4, or pH 5.6. What is true of the dilute gluten solutions was also found to apply to the natural gluten as recovered from flour dough. Gluten, therefore, appears to be something other than a simple mixture of gliadin and glutenin, but its existence is held to be dependent on an interaction between the components.

When gluten from a strong and a weak flour were compared, the maximum turbidity of the solutions of these two glutes passed through a maximum in the same range of pH when the latter was varied. Moreover, the actual magnitudes of turbidities of solutions of the two glutes were almost the same at all points when the quantity of protein in the two preparations was the same.

Since the non-gluten proteins of wheat flour have an isoelectric point at a lower pH value than gliadin, they must be negatively charged at the pH where a simple gliadin-glutenin complex would be most completely formed. Therefore the point of maximum complex formation between gliadin and glutenin will be influenced by the presence of these substances, and will occur at lower pH values as a function of their concentration. Thus with a low-grade flour, containing more non-gluten proteins than the patent flour milled from the same wheat, the turbidity curve with varying pH will be different from that of the patent flour. This will take the form of a curve shifted in the direction of maximum turbidity at a lower pH value.

Thus we find in this concept of coacervation or mutual coagulation a more adequate basis for accounting for the relations of gliadin to glutenin in the gluten complex than had hitherto been proposed. The type of diagram employed by Schmorl⁵⁸ for the space configuration of a typical protein molecule facilitates an understanding of the possible role of the polar groups of such molecules in their contribution to the electrostatic attraction of different molecules for one another, and the behavior of such substances in the processes of hydration.

In a discussion of the swelling of gluten in water Kul'man⁵⁹ contends that it is chiefly osmotic in nature and depends on the degree of aggregation of the fractions composing it. Using the heat of hydration as a criterion, it appears that the small difference between gliadin and glutenin

⁵⁸ *Z. ges. Getreidew.*, **25**, 144 (1938).

⁵⁹ *Colloid J. (U.S.S.R.)*, **3**, 863 (1937); *Chem. Abs.*, **32**, 7063 (1938). Original not seen.

in that characteristic indicates that they have the same relative amount of hydrophilic groups. They do differ in the amount of swelling and water absorption, however; this is interpreted to imply a difference in the dimensions and structure of their micelles.

Gliadin and glutenin are characterized by Gershzon⁶⁰ as being hydrophobic externally and hydrophilic internally, which is thought to explain their capacity to absorb water and swell. The pressure of swelling of air-dried flour and of its proteins (moisture content about 10 per cent) was found to be approximately 50-70 atmospheres.

⁶⁰ *Colloid J. (U.S.S.R.)*, 3, 897 (1937); *Chem. Abs.*, 32, 7063 (1938). Original not seen.

Chapter 6

Nucleic Acid

Nucleic acid of the wheat embryo was recovered by Osborne and Campbell¹ from a pepsin digest of a sodium chloride solution extract. The nuclein recovered was washed with dilute KOH, and then treated with dilute HCl until a precipitate formed. This was filtered off, and treated with strong HCl. The flocks were redissolved in alkali, and reprecipitated with acid. Finally it was dissolved in alkali and the solution poured into alcohol. The latter retained the coloring matter, while the potassium nucleate settled out. The latter was washed with alcohol, dried, dissolved in water, and the nucleic acid precipitated by an excess of HCl. The average composition, computed to a potassium metaphosphate-free basis, was

Carbon	36.48	per cent
Hydrogen	4.48	" "
Nitrogen	16.17	" "
Phosphorus	8.96	" "
Oxygen	33.91	" "

Guanine and adenine were identified in and recovered from an HCl hydrolyzate. No sugar was identified. Their formula for the nucleic acid of wheat germ then became $C_{53}H_{77}N_{20}P_5O_{37}$.

About 3.5 per cent of the commercial wheat embryo examined by Osborne and Harris² consisted of tritico-nucleic acid, which had the properties of nucleic acids of animal origin, but was less soluble in water. Its composition corresponded to the formula $C_{41}H_{61}N_{16}P_4O_{31}$. With potassium, sodium, and ammonium hydroxide it formed acid salts, which gave an acid reaction to litmus in their water solution. It accordingly was impossible to prepare a base-free acid.

Hydrolysis with acid gave 1 molecule of guanine, 1 of adenine, 2 of uracil, and 3 of pentose for each 4 atoms of phosphorus. On the basis of its reaction with silver salts, the acid appeared to have 6 free acid hydroxyl groups. A complicated ester was indicated, of penta-hydroxyphosphoric acid, unknown in the free state, but yielding stable esters. On hydrolysis

¹ Conn. Agr. Exp. Sta. 23rd Annual Report for 1899, p. 305 (1900).

² *Z. physiol. Chem.*, 36, 85 (1902).

with dilute acids, considerable guanine and adenine were split off, together with about one-fourth of the phosphorus as orthophosphoric acid.

In addition to the uracil previously obtained by Osborne and Harris,³ cytosine was recovered and identified by Wheeler and Johnson.⁴

Nucleic acid from the wheat embryo was found by Osborne⁵ to be strongly dextrorotatory; values from $+67^{\circ}$ to $+73^{\circ}$ were obtained, the variation being a function of the concentration. This property of the nucleic acid doubtless accounted for the unexpected property of dextro-rotation of the nucleo-proteins.

The question whether cytosin was a primary or a secondary product of the cleavage of nucleic acids was raised by Burian.⁶ He had demonstrated experimentally the possibility of its appearance as a derivative of other purines in the presence of decomposition products of carbohydrates. Uracil was also observed to appear when nucleic acids were treated with HNO_3 , and its actual origin remained in doubt.

Since Burian had raised the question as to whether the uracil and cytosine, which appear when nucleic acids are subjected to severe hydrolysis, actually exist preformed in the molecule, or are formed from the purines under the conditions of hydrolysis, Osborne and Heyl⁷ conducted a further study involving mild hydrolysis to remove the purines. Accordingly, tritico-nucleic acid was boiled with 2 per cent H_2SO_4 (by vol.) for two hours, and the purines precipitated as silver salts by adding Ag_2SO_4 . On decomposition and suitable manipulation, cytosine and uracil were recovered and identified in the ratio of 1 mol.:1 mol.

From these and earlier results of Osborne and Harris,⁸ they concluded that 15/16 of the nitrogen of the nucleic acid probably belongs to guanine, adenine, cytosine and uracil, of which one molecule of each is present for every four atoms of phosphorus. The other 1/16 was not identified at the time.

Tritico-nucleic acid, recovered from wheat germ by Levene and La Forge,⁹ resembled the nucleic acid which they had previously prepared from yeast. The pentose was evidently *d*-ribose; guanidin, adenine, and cytosine were also identified.

Purine bases (xanthine, hypoxanthine, guanin and adenine collectively) were determined by Fellenberg¹⁰ in a large variety of food materials. In wheat and its products the following total purine content was reported, all results being calculated to the dry basis: Entire wheat, 0.070 per cent;

³ *Z. physiol. Chem.*, **36**, 85 (1902); Annual Rpt. Conn. Agr. Exp. Sta. for 1901, p. 408.

⁴ *Am. Chem. J.*, **29**, 505 (1903).

⁵ *Am. J. Physiol.*, **9**, 69 (1903).

⁶ *Z. physiol. Chem.*, **51**, 438 (1907).

⁷ *Am. J. Physiol.*, **21**, 137 (1908).

⁸ *Z. physiol. Chem.*, **36**, 85 (1902).

⁹ *Ber.*, **43**, 3164 (1910).

¹⁰ *Biochem. Z.*, **88**, 323 (1918).

"full flour" of 92 per cent extraction, 0.054 per cent; flour (extraction not given), 0.015 per cent; wheat bran, 0.105 per cent. These purines were recovered as their copper salts, after the copper sulfate-bisulfite method of Krüger and Schittenhelm, with suitable modifications to adapt it to substances low in purine content.

Nucleic acid from wheat germ was studied by Thomas and Dox¹¹ in the form of various salts with bases. The finding of 7 or 8 sodium atoms in several preparations led them to the conclusion that the individual nucleotides in the nucleic acid are bound in ester combinations.

Further reference to the nucleic acid and nuclein content of wheat products will be found in the section on phosphorus in the chapter on minerals.

¹¹ *Z. physiol. Chem.*, **142**, 1 (1925).

Chapter 7

Non-Protein Nitrogen

It has long been apparent that practically all the nitrogen of flour, as determined by the conventional Kjeldahl method or one of its modifications, was derived from simple proteins. Moreover, the non-protein nitrogen of flour has not been regarded by flour technologists as singularly significant, except in the instance of the attempts that have been made to estimate the rate of proteolysis from the progressive appearance of non-protein nitrogenous compounds in autolytic digests, or extracts of doughs and batters. Consequently much of the literature on this phase of the studies of nitrogenous constituents has been related to observations of proteolysis. Moreover, these studies have been somewhat disappointing, to the extent that it is obvious that profound changes may occur in the properties of gluten proteins which have a major bearing upon the significant physical properties of dough, and yet with modest increases in the amino and other simple, non-protein constituents.

Certain exceptions to these generalities are noted, however, in the following paragraphs of this chapter.

Stutzer's ¹ method involving the precipitation of proteins with cupric hydroxide was modified by him ² to include the addition of alum solution before applying the copper reagent. This modification was studied by LeClerc and Lounsberry,³ who found that less protein was precipitated when alum was present in the reaction mixture, and concluded that it should be omitted.

The use of Stutzer's cupric hydroxide reagent did not prove entirely satisfactory in the hands of Teller,⁴ who preferred phospho-tungstic ("phospho-wolframic") acid for precipitating the proteins in determining non-protein or "amid" N in wheat. In a series of Arkansas mill flours, ranging from patent (0.41 per cent ash), to ship stuff (0.71 per cent ash) the amid nitrogen ranged from 0.03 to 0.05 per cent of the flour. In another series the quantity of amid nitrogen was greater, representing from 0.05 to 0.09 per cent. In a set of hard winter wheats the range was from 0.07 to 0.12 per cent, averaging 0.09 per cent; in three spring wheats he found 0.17, 0.09 and 0.11 per cent respectively.

¹ *J. Landw.*, 28, 103 (1880).

² *J. Landw.*, 34, 151 (1886).

³ U. S. Dept. Agr., Bur. Chem. Bull. 99, 150 (1906).

⁴ *Ark. Agr. Exp. Sta. Bull.* 42 (1896).

In an extension of these studies Teller⁵ applied the same method to the determination of the amid-nitrogen content of wheat kernels gathered on 42 consecutive days from the time of setting to full ripeness. On the 1st day the kernels contained 0.67, on the 42nd day 0.08 of amid nitrogen. The most rapid rate of change was between the first and the eighth day, when it was 0.21 per cent; the change was gradual thereafter, reaching 0.10 per cent on the 19th day.

Amid nitrogen, as determined after precipitating the proteins from an aqueous extract with phosphotungstic acid, was found by Teller⁶ to constitute 0.10 per cent of the wheat kernel, 0.53 per cent of the germ, 0.19 per cent of the bran, and 0.03 per cent of the flour.

The Sorenson formol titration method was applied to aqueous extracts of ground whole wheat by Jodidi and Markley.⁷ They endeavored to remove as much of the protein from the extract before titration as was possible, because of the extent to which the free amino groups of the proteins are actually involved in the titration. On the dry basis the amino nitrogen thus determined was in the range 0.033 in two samples of whole wheat, to nearly double that figure, or 0.060, in two other samples. Polypeptides were determined also, by treating the protein-free extract with magnesium oxide and distilling to remove the NH_3 , and then Kjeldahling the residue. Upwards of 5 per cent of the total nitrogen of the wheat was assumed to be in the peptide form.

Asparagin, allantoin, choline, and betain were identified in wheat germ by Frankfurt,⁸ who reported a total of 0.80 per cent of amid nitrogen on the dry basis.

Allantoin was identified in the wheat grain by Fosse, Brunel, de Graeve, Thomas and Sarazin,⁹ but they do not indicate the quantity that was present. Their method for the identification of this substance was given in detail.

Betain was encountered in wheat bran by Nottbahrn and Mayer¹⁰ in a concentration of about 0.35 per cent.

The small proportion of non-protein nitrogenous compounds in the entire wheat kernel was emphasized by Schulze and Castoro,¹¹ who quote Wolff-Lehmann's data as follows:

Wheat 0.240 per cent = 11.2 of the total nitrogen.

In the wheat embryo they identified asparagin, allantoin, choline, betain and arginine.

⁵ *Ark. Agr. Exp. Sta. Bull.* 53 (1898).

⁶ *Proc. 8th Internat. Cong. Appl. Chem.*, VIa, 273 (1912).

⁷ *J. Am. Chem. Soc.*, 45, 2137 (1923).

⁸ *Landw. Vers. Sta.*, 47, 449 (1896).

⁹ *Compt. rend.*, 191, 1153 (1930).

¹⁰ *Z. Untersuch. Lebensm.*, 69, 301 (1935).

¹¹ *Z. physiol. Chem.*, 41, 455 (1904).

The reagents commonly used for precipitating proteins, including alcohol, acetic acid, trichloroacetic acid, salts of heavy metals, colloidal iron, aluminum hydroxide cream, phosphotungstic acid and tannic acid were regarded by Blish¹² as being unsatisfactory for removing gliadin from water extracts of flour. He then proceeded to modify the application of the Stutzer reagent by forming the cupric hydroxide in the extract itself. This was done by treating the extract with 0.1N NaOH, and then adding 0.1N CuSO₄ until but slightly more CuSO₄ was present than the equivalent NaOH first added. Some peptide nitrogen was not precipitated by the resulting Cu(OH)₂, but the removal of true proteins was regarded as practically complete. Sound or normal patent flour was found to contain about 2 mg of amino-acid nitrogen per 100 g of flour, and about three times as much nitrogen in free acid-amide form when the latter was determined by König's method.¹³

Blish's method for precipitating the flour proteins from an aqueous extract was modified by Olsen and Bailey,¹⁴ who controlled the addition of the requisite quantity of copper sulfate by adding phenolphthalein with the NaOH solution, and then the CuSO₄ until the original purple tint changed through a blue to a green hue. Since the level of pH is a critical factor, the control thus afforded insured that the removal of the alkali by its conversion into insoluble Cu(OH)₂ was complete.

Cairns and Bailey¹⁵ compared seven methods for the determination of non-protein nitrogen including (1) Blish's modification of Ritthausen's method using cupric hydroxide; (2) Schwering's method with stannous chloride; (3) Sörensen's formol titration; (4) Van Slyke's amino-acid method; (5) the trichloroacetic acid method as applied by Hiller and Van Slyke; (6) precipitation of proteins with sodium tungstate; (7) Foreman's titration in alcohol. The Sörensen method was preferred for measuring proteolysis; the Van Slyke method was useful, though more laborious.

Using phosphotungstic acid as a protein precipitant, Sharp and Elmer¹⁶ found only 0.03 to 0.05 per cent of amino-nitrogen (expressed as protein N \times 5.7) in the flours at the outset of a period of auto-digestion in water suspensions. When these suspensions were incubated at 35° C for 5 weeks the amino-nitrogen increased progressively, reaching the level of 0.45 to 0.49 per cent at the end of the period.

The same investigators also traced the changes in the percentage of the total nitrogen represented in the amino-nitrogen fraction of wheat

¹² *J. Biol. Chem.*, **33**, 551 (1918).

¹³ "Chemie der menschlichen Nahrungs und Genussmittel III," Berlin, p. 274 (1910).

¹⁴ *Cereal Chem.*, **2**, 68 (1925).

¹⁵ *Cereal Chem.*, **5**, 79 (1928).

¹⁶ *Cereal Chem.*, **1**, 83 (1924).

kernels from the 13th to the 33rd day of their development in the head. This level, thus recorded, fell substantially from the 13th to the 20th day and then more slowly thereafter. While the graphic records cannot be read very exactly, the total change during the period appears to have been from about 7 per cent of the total nitrogen to less than 1 per cent.

Another method was developed by Sharp¹⁷ for recovering and estimating the amino-acids of wheat during maturation. The kernels were threshed and at once thrown into boiling water. After 20 minutes of boiling they were disintegrated in a mortar, retreated with hot water, and the proteins in this digest were precipitated with sodium tungstate in an acidulated solution. Then the amino-acids in the solution were determined in a Van Slyke micro apparatus, and expressed as amino-nitrogen $\times 5.7$. In spring wheat kernels 4 days after kernel formation had started, when the kernels weighed 2.7 milligrams (dry), 19.6 per cent of the nitrogen was in the amino form. At the 10th day, when the kernel weight was 6.5 mg, it was 10.8 per cent; two weeks later, when the kernel weight was 25.2 mg, it was 4.7 per cent; at maturity it had fallen to 0.4 per cent. Freezing of immature kernels evidently arrested or interfered with the conversion of simple amino-compounds into proteins, the degree of difference being a function of the relative immaturity of the kernels. In a subsidiary study of the amino-nitrogen determination by the Van Slyke methods, the largest yields of nitrogen gas were obtained when the aqueous extract of the kernels was treated with SnCl_2 (I) in alcohol and made to volume with alcohol; the smallest yield of about 70 per cent as much nitrogen gas as in (I) was recovered from the use of 7 cc (II) of 15 per cent sodium tungstate solution per 100 cc of final volume, whereas when 2 cc (III) of this reagent was used as the protein precipitant, the yield of gas averaged 92 per cent of that recovered from (I). From his comments it would appear that Sharp preferred method II, or an approach to it which gave the lowest results.

The nitrogen which was non-precipitable by the tin and the copper reagents was determined by Sherwood and Bailey¹⁸ in flours to which varying percentages of sprouted wheat had been added. The values were lowest with the copper reagent, used as described by Olsen and Bailey,¹⁴ and the normal flour contained 0.020 per cent of nitrogen not precipitated by it. When 10 and 40 per cent of wheat germinated for 3 days was included in the mill mixture the non-precipitable nitrogen in the flour was 0.032 and 0.038 per cent respectively; with wheat germinated for 5 days the corresponding percentages were 0.025 and 0.053 per cent. Inter-

¹⁷ *Cereal Chem.*, 2, 12 (1925).

¹⁸ *Cereal Chem.*, 3, 107 (1926).

mediate values were found with smaller percentages of germinated wheat.

As has been emphasized, many of the studies of non-protein nitrogen in wheat products have involved consideration of the rate of increase in such constituents with the lapse of time when doughs or other preparations were fermented. This was true in the researches of Olsen and Bailey,¹⁴ Cairns and Bailey,¹⁹ Brownlee and Bailey,²⁰ and Samuel.²¹ The latter, employing a modification of the Sörensen formaldehyde titration, found the following equivalents of amino-acid nitrogen in milligrams per gram of flour, when freshly mixed with water:

Flour 1.	A mill mixture	0.07
Flour 2.	A mill mixture	0.085
Flour 3.	Manitoba	0.11
Flour 4.	Low grade	0.175
Flour 5.	English	0.16

In his study of methods for determining amino-acids in flour, using Brown's modification of the Sörensen formal titration, Samuel²¹ found that extraction was complete in one hour, and thereafter for a few hours there was no substantial increase in the amino-acid content of the extract. This does not imply, to be sure, that there may not be substantial changes in the physical properties of the native proteins of the flour during these extended extraction periods, but these changes are not manifested by the increase in amino-acid nitrogen. The quantity of the latter encountered in several flours was of the same general level of magnitude as was reported in Samuel's²² earlier paper, *i.e.*, 15 to 20 milligrams of amino-acid nitrogen per gram of flour. His observations did not support the earlier work of Denham and Scott Blair,²³ who had found that the amino N content of a wheat extract increased as a function of the logarithm of the time of extraction, and had proposed that the true amino-acid content could be estimated by extrapolating the recorded values to zero time. On this basis they estimated the initial amino-acid content, calculated as glycine, in three wheats as follows: A typical mill mixture, 0.203 per cent; a Manitoba wheat, 0.196 per cent, and an English wheat as 0.229 per cent.

The 1941 edition of *Cereal Laboratory Methods* edited by a committee of the American Association of Cereal Chemists includes a method (Chap. III, Sect. 9) for the determination of amino-acids in flour involving the Sörensen method. A 20-g charge of flour is suspended in 100 ml of water, with intermittent shaking for 1 hour. The extract is recovered by centrifuging and filtering, and to 10 ml is added sufficient *N*/14 NaOH to bring the pH to 8, as shown by the color change in phenol red used as an indi-

¹⁹ *Cereal Chem.*, **5**, 79 (1928).

²⁰ *Cereal Chem.*, **7**, 487 (1930).

²¹ *Biochem J.*, **29**, 2331 (1935).

²² *Biochem. J.*, **28**, 273 (1934).

²³ *Cereal Chem.*, **4**, 158 (1927).

cator. The exact quantity of the standard HCl is recorded, and this represents the titratable acidity of the extract (I). Add additional indicator, plus 8 ml of 40 per cent formaldehyde, and again titrate with $N/14$ NaOH (II). An equivalent amount of the formaldehyde solution, diluted with water, is also titrated, and this constitutes a blank (III). This blank III plus the titratable acidity I are subtracted from the titration II and calculate the difference as mg amino-N per 100 g flour.

In a series of four flour samples representing as many commercial grades, Johnson, Herrington and Scott²⁴ found the amino-acid content to increase with the ash content, as shown by the data in Table 49. The amino-acid in the fresh extract was determined by both the Sörensen formol titration and the Van Slyke method. The latter gave consistently lower results.

Table 49. Ash and Amino-nitrogen Content of Flour Grades, as Determined by Johnson, Herrington, and Scott.²⁴

Grade	Ash (%)	Amino N by formol titration (mg per 10 g)	Amino N by Van Slyke method (mg per 10 g)
Patent	0.41	0.45	0.31
Straight	0.62	0.60	0.38
1st clear	0.93	0.75	0.50
2nd clear	1.88	1.70	1.08

In a study of flour grades, Swanson and Tague²⁵ observed that "titratable nitrogen," i.e., the nitrogen in an aqueous extract as determined by the Sörensen formol titration, is more uniformly distributed in the wheat kernel than are the materials which determine the amount of ash and acidity. Hence the titratable nitrogen does not increase regularly with the percentage of ash.

A steady decrease in the asparagin nitrogen in wheat during the growing period from June 13, when the average kernel weight was 9.17 mg, to August 9, when it was 45.46, was observed by Nedokutschajew²⁶ of the Agricultural Institute of Moscow. He used Sachsse's method for the determination, and the values were 0.29 and 0.11 per cent at the two stages. He also computed the amid nitrogen by the difference between total N, and protein N as determined by Stutzer's method and found it to be 0.68 per cent on June 13 and 0.13 per cent on August 9.

Microchemical techniques were applied by Eckerson²⁷ to wheat kernels collected at various stages of maturity. She found much asparagine, arginine, histidine and leucine in freshly gathered immature wheat kernels. No protein reaction was obtained then, but if the grains were permitted to

²⁴ *Cereal Chem.*, **6**, 182 (1929).

²⁵ *J. Am. Chem. Soc.*, **39**, 482 (1917).

²⁶ *Landw. Vers.-Sta.*, **56**, 308 (1902).

²⁷ *Wash. Agr. Exp. Sta. Bull.* 139 (1917).

dry gradually for 12 hours, a strong protein reaction was secured, and gluten could then be identified, while the arginine, histidine and leucine disappeared and only a trace of asparagin remained. Eckerson's observations were supported by the parallel chemical studies conducted by Olson.²⁸

The percentage of the total nitrogen of the developing wheat kernel that was present in the amid fraction as separated by the Stutzer reagent was shown by Thatcher²⁹ to decrease steadily as maturation approached. Thus in spring wheat kernels analyzed 8 days after blossoming, 23.1 per cent of the nitrogen was in the amid fraction, while 18 days later when the kernels were fully developed the amid nitrogen fraction was only 5.9 per cent of the total nitrogen.

Wheat which was harvested at three different stages of maturity by C. O. Swanson *et al.*³⁰ and described as (1) under-ripe, (2) prime condition, and (3) over-ripe contained 0.463, 0.414 and 0.381 per cent of "amino-compounds" respectively, on a moisture-free basis. The method used for the determination of amino-compounds is not described, but presumably it represented the nitrogen in an aqueous extract as determined by formol titration as employed by Swanson and Tague,³¹ who reported a detailed study of proteolysis in wheat flour. There was a slight increase in the amino compounds in lot 2 in the stack, or shock, after 5 weeks; also an increase to 0.479 per cent when moistened to 13.54 per cent and stored in a bin for 10 weeks.

Wheat tempered with varying amounts of water, held at different levels of temperature from 45 to 98° C for various lengths of time, and then milled into flour, exhibited a tendency toward increasing percentages of amino compounds as a function of (a) quantity of water added, (b) temperature, and (c) time, although the relation is erratic. Certain typical data include the following:

Tempering conditions	Amino-compounds in flour after tempering for			
	3 hrs	6 hrs	12 hrs	24 hrs
25 cc water at 45° C	0.210	0.208	0.200
50 cc water at 45° C	0.220	0.198	0.243
22 cc water at 70° C	0.226	0.223	0.211	0.226
50 cc water at 70° C	0.241	0.225	0.256
25 cc water at 98° C	0.237	0.306

These investigators also subjected wheat to conditions promoting germination, then dried the wheat after varying intervals of time, and milled it into flour. In the first trial, flour from ungerminated wheat contained 0.145 per cent of amino compounds, and after 130 hours' germination it had increased slightly to 0.164 per cent. This first series gave very

²⁸ Wash. Agr. Exp. Sta. Bull. 142 (1917).

²⁹ J. Am. Soc. Agron., 7, 273 (1915).

³⁰ Kans. Agr. Exp. Sta. Tech. Bull. 1 (1916).

³¹ J. Am. Chem. Soc., 38, 1098 (1916).

erratic results, however. In a second series, the ungerminated wheat flour contained 0.093 per cent of amino compounds, and there was a steady and regular increase in the latter with lapse of time in germination, until at the 119th hour it had reached 0.376 per cent.

Delayed harvesting of winter wheat grown at Nephi, Utah, for periods as long as two months after ripening, did not result in any substantial increase in the non-protein nitrogen, as determined by Bracken and Bailey.³²

The percentage of amino nitrogen in flour milled from wheat harvested in the "dough" stage (I) was not greatly different from the flour milled from normally (II) or dead-ripe wheats (III) in the experiments reported by Mangels and Stoa.³³ With the Sörensen formol titration, the values were 0.009 per cent amino acid for I, 0.008 for II; with tungstic acid as the protein precipitant, the non-protein nitrogen was 0.027 per cent in I, and 0.020 per cent in II and III.

Frosted wheats contained more non-albuminoid nitrogen than sound wheats as determined by Shutt.³⁴ He used the Stutzer reagent to precipitate the "albuminoids." Sound wheat was found to contain 0.08 to 0.14 per cent non-albuminoid nitrogen, frosted wheat 0.06 to 0.23 per cent, and badly frosted wheat 0.21 to 0.58 per cent. This large difference in the wheats did not persist in flours milled from them, however. Thus, in two sound wheat flours there was 0.03 and 0.10 per cent, and in three frosted wheat flours 0.03, 0.04 and 0.06 per cent. Shutt concluded that the non-albuminoid constituents of frosted wheat must reside in, or be associated with the embryo, and are thus separated in the process of milling.

Wheat frozen while still immature contained substantially larger proportions of their total nitrogen in non-protein combinations in the studies conducted by Blish.³⁵ Two series of investigations were conducted by him in 1917 and 1918, respectively. In Table 50 appear the results of these non-protein nitrogen determinations as applied to the wheat samples, and to flours milled from them. In the 1917 series the analyses of the

Table 50. Non-protein Nitrogen as Percentage of Total Nitrogen in Frosted Wheats, and Flours Milled from Them, as Determined by Blish.³⁵

Description	Non- protein N in total N	
	Wheat (%)	Flour (%)
1917 Frozen when mature	4.2	1.8
1917 Frozen when immature	7.0	4.4
1917 Frozen in late milk stage	14.0	10.6
1918 Frozen when mature	7.7	3.0
1918 Frozen when slightly immature	10.7	3.6
1918 Frozen in early dough stage	13.2	5.1

³² *Cereal Chem.*, 5, 128 (1928).

³³ *Cereal Chem.*, 5, 385 (1928).

³⁴ Report of the Exp. Farms (Canada) for 1906, p. 140 (1907).

³⁵ *J. Agr. Res.*, 19, 181 (1920).

flours paralleled those of the wheat, but in 1918 the differences between the flours were not so great. The latter has been the more general experience in studies of this type.

The Sullivan and Hess³⁶ test for free cysteine was applied to wheat germ by Sullivan, Howe and Schmalz³⁷ with negative results, but they obtained a positive test for glutathione. Using the iodine titration method of Weller,³⁸ they found 0.46 of glutathione in freshly milled wheat germ, and they succeeded in actually recovering 0.26 g of impure glutathione from one kilo of germ (dry basis).

The presence of substances containing S-H groups, such as glutathione, and possibly certain proteins and protein split-products, is assuming a large significance at this time. Generally speaking, this S-H group is being assigned a substantial role in: (a) its relation to the level of activity of certain proteinases, and (b) its direct effect upon the physical properties of dough. The oxidation-reduction system of flours and doughs bids fair to assume a position of major importance in the study of baking qualities, but at this time our knowledge is too incomplete to permit of a discussion of its quantitative aspects, or of the substances which contribute most prominently to the oxidation-reduction potential.

In the germ of wheat of the Sinvalochó variety grown in Argentina, Albizzati³⁹ found 0.345 per cent of glutathione.

A method was proposed by Freilich⁴⁰ for determining the reducing matter in flour, involving the precipitation of the proteins from an extract of flour in dilute saline solution, addition of a unit volume of standard iodine solution, and back-titration of the residual iodine with standard thiosulfate solution.

With a quantity of saline extract apparently equivalent to 100 g of flour, the reducing matter present, in terms of milliliters of 0.01*N* iodine used, in a series of flours of different grades milled from Northwestern wheat was:

Patent flour	0.9
Straight grade flour	1.2
Clear flour	1.5
Low grade flour	3.5

Similar results were obtained with flours milled from Texas wheat, except that the value for the low-grade flour was only 1.9 cc. In both series there was a definite trend toward an increase in reducing matter with a diminishing degree of refinement of the flour.

³⁶ *U. S. Public Health Reports*, 46, 390 (1931).

³⁷ *Cereal Chem.*, 13, 665 (1936).

³⁸ *Ann. ferm.* (1935) 108.

³⁹ *Anales soc. cient. Argentina*, 124, 194 (1937).

⁴⁰ *Cereal Chem.*, 18, 128 (1941).

Chapter 8

Starch

The principal constituent of the endosperm of wheat, and hence of flour, in point of quantity, is starch. In the normally ripened wheat grain, and in natural flour, starch occurs in the form of granules (Fig. 7) of more or less characteristic forms and sizes, having a specific gravity of about 1.5. Three general sizes of granules can be distinguished by microscopic examination: (1) large granules over $15\ \mu$ in diameter and ranging up to $30\text{--}35\ \mu$, or occasionally even larger; (2) intermediate sizes between

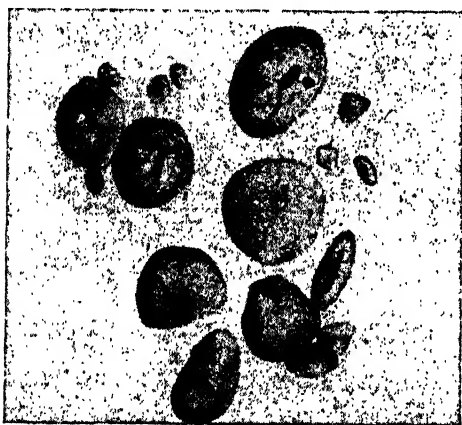


FIG. 7. Photomicrographs of typical wheat starch granules. (From U. S. Dept. Agr. Bur. Chem. Bull. 13, Pt. 9)

7.5 and $15\ \mu$ in diameter; (3) small granules less than $7.5\ \mu$ in diameter. In a series of 15 flour samples of *vulgare* wheat flour examined by Grewe and Bailey,¹ the larger sizes of granules constituted an average of about 12.75 per cent by count (not by weight), the intermediate sizes about 6 per cent by count and the small granules made up the remainder. There was considerable variation from sample to sample in the first two classes of sizes, however. Stamberg² calculated the percentage by weight of each size and found 4.1 per cent of the small, 2.9 per cent of the intermediate, and 93.0 per cent of the large granules. The relative surface area of the granules in these flours was estimated by him to be 17.7, 5.9 and 76.4 per cent respectively for these three groups of small, intermediate, and large starch granules.

¹ *Cereal Chem.*, 4, 230 (1927).

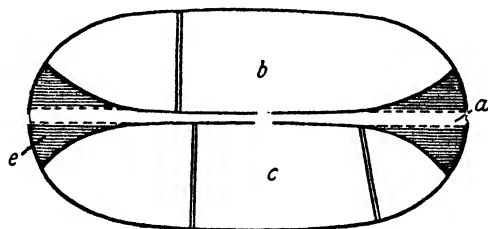
² *Cereal Chem.*, 16, 769 (1939).

STARCH

No substantial difference in the total phosphorus content of large and small granules separated from the same original flour sample could be detected by Stamberg and Bailey.³ Nor was there any evidence of a difference in the ratio of amylopectin to amylose when these constituents were fractionated by the electrophoretic technique employed by them.

While the small starch granules appear to be more or less spherical in shape, the large granules actually are oblate spheroids or discs, distinctly flattened on the poles. Badenhuizen⁴ presented the diagram of Nikolaieff and Schultz⁵ reproduced in Fig. 8, which indicated the relative dimensions of the area of cross-section. This diagram also indicated three gen-

FIG. 8. Scheme of structure of a wheat starch granule, after Nikolaieff and Schultz (5); a. middle plate; b. upper half; c. lower half; e. a fan-like spreading of the middle plate at the edges.



eral types of structures in the granule, (a) the middle plate, (b) and (c) the upper and lower halves, and (e) a fan-like spreading of the middle plate at the edge. Reference will be made later to the distribution of different materials in the granules.

Heat treatment of such granules in water suspension, followed by careful mechanical manipulation, results in a separation of an envelope or sac, the so-called starch membrane, from the inner contents. In fact, this had been observed by Leeuwenhoek as early as 1716. The evidence of such a membrane is presented in elegant fashion by Badenhuizen.⁶ In another paper Badenhuizen⁷ described methods of micro-manipulation employed to demonstrate that the wall of a swollen granule arises from the confluence of the resistant layers in the original starch granule. Thus the granule really consists of a series of sacs enveloping one another. The exterior envelope is not regarded by him as a special membrane, and the assumption that it takes a different color with iodine was found to be incorrect when a stained granule was halved.

Severe over-grinding, as with a ball or rod mill, tends to disintegrate the envelopes and render the contents more readily "soluble" than in the instance of the intact, unground granules. Dry granules, on suspension in cold water, absorb a small amount of water. If the temperature of the

³ *Cereal Chem.*, **16**, 309 (1939).

⁴ *Protoplasma*, **28**, 312 (1937).

⁵ "The Microstructure of Bread," Research Inst. Baking Industry, U.S.S.R. (1933).

⁶ *Rec. trav. botan. neerland.*, **35**, 559 (1938).

⁷ *Chron. Bot.*, **4**, 123 (1938).

water is elevated, a substantial increase in water imbibition occurs as the temperature passes through a certain range.

Reichert⁸ records the swelling and gelatinization temperatures of a number of starches, including wheat; for the latter, swelling began at 50°, gelatinization began at 65° and was complete at 67.5°. Sjöström's⁹ photomicrographs of wheat starch granules "pasted" at about 65° indicated an increase in volume of the order of ten-fold or thereabouts. There is a large increase in the "viscosity" of the heterogeneous suspension as well. The data of Alsberg and Rask¹⁰ indicated that the viscosity of 4½-5 per cent wheat starch suspensions did not increase substantially until the temperature exceeded 90°, and reached a maximum at about 95°.

The beginning of a significant swelling of individual wheat starch granules was observed by Katz and Hanson¹¹ at 50°, although gelatinization was not in evidence until 65° was reached. Disappearance of the granule forms was not complete until the gel was heated to 67°.

The assumption repeatedly made by earlier investigators that the envelopes of the granules are uniformly ruptured by such thermal treatment is evidently in error. Simple microscopic examination of a heated paste did not always clearly disclose the boundaries of the greatly swollen granules, even when they were essentially intact. The more careful work and superior micro-techniques applied to recent studies have facilitated the tracing of the swelling process.

In addition to the increase in viscosity of starch suspensions effected by heating, the treatment of starch with various reagents, including solutions of sodium hydroxide, sodium salicylate, thiocyanates, iodines, and urea will likewise effect substantial increases in viscosity. Mangels and Bailey¹² found that *durum* wheat starch exhibited a greater swelling capacity when treated with NaOH and urea solutions than did starches from the common or *vulgare* varieties. No such difference between *durum* and hard winter wheat starch was observed on treatment with thiocyanate, iodide and sodium salicylate solutions, but hard spring wheat starch showed less response.

Mangels¹³ confirmed the observation respecting the difference in behavior of *durum* and *vulgare* varieties with NaOH (0.1M). He further noted that, in general, the starch in wheat grown in hot, dry seasons was less susceptible to the action of swelling reagents than when the grain is grown in seasons of lower temperature and more abundant rainfall. He

⁸ "The Differentiation and Specificity of Starches in Relation to Genera, Species, etc.," Part 1, pp. 174-75 (1913).

⁹ *Ind. Eng. Chem.*, 28, 63 (1936).

¹⁰ *Cereal Chem.*, 1, 107 (1924).

¹¹ *Z. physik. Chem. [A]*, 168, 321 (1934).

¹² *Ind. Eng. Chem.*, 25, 456 (1933).

¹³ *Cereal Chem.*, 11, 571 (1934).

also noted an inverse ratio between phosphorus content and swelling capacity of wheat starch granules.

In his last paper on this subject Mangels¹⁴ laid further emphasis upon the varietal and regional effects upon *durum* starches, and observed that low phosphorus content may be associated with high susceptibility to diastase, although this relationship is not uniformly consistent.

Referring back to the native untreated starch granules, it has long been known that striations representing more refractory zones could be discerned in many kinds of starches when observed with a compound microscope. More refined studies, after controlled swelling, and through the use of stains, revealed other details of structure. These have led to such conclusions as those of Hanson and Katz,¹⁵ that the granule is constructed of blocklets about $1\ \mu$ across and arranged as shown in Fig. 9. Viewed from the side, these blocklets would appear as in Fig. 10. Other

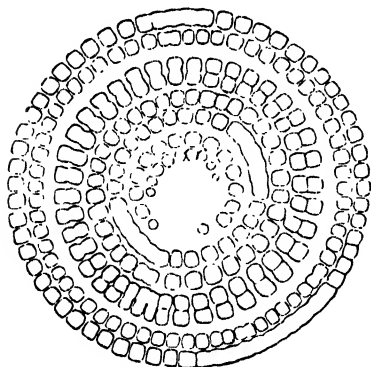


FIG. 9. Individual blocklets in a wheat starch granule after "Lintnerizing" for $1\frac{1}{2}$ weeks and then swelling in $2M\ Ca(NO_3)_2$ solution. [After Hanson and Katz (15)]

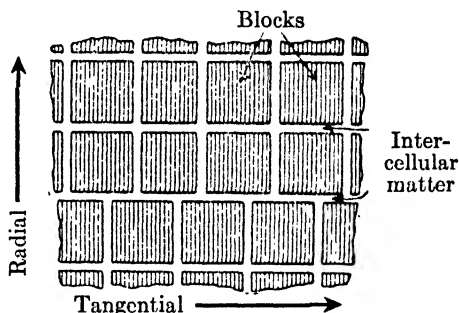


FIG. 10. Scheme of the blocklet structure of a starch granule. [After Hanson and Katz (15)]

workers have assigned various names to essentially identical structures, such as "micelles," "micro crystals," or "trichites." Sjostrom's⁹ photomicrographs show similar characteristics, and he apparently accepts the structural concept of Hanson and Katz.

Sande-Bakhuyzen¹⁶ could not discern any lamellation or rings in the starch granules separated from wheat grown under constant illumination and physical environment, however. When the starch was heated to the temperature where the granules began to swell, he could see refractive, radial needles, with a base of $2\text{--}3\mu$ and tapering toward the center to a

¹⁴ *Cereal Chem.*, **13**, 221 (1936).

¹⁵ *Z. physik. Chem.*, **163**, 339 (1934).

¹⁶ *Proc. Soc. Exp. Biol. Med.*, **23**, 302 (1926).

length of 17-20 μ . While the lamellated granules from an ordinary wheat kernel also showed needles, they extended only to the first non-refractive ring, a distance of about 5 μ , or 10 \pm per cent of the diameter of the granule. On the basis of earlier work, Sande-Bakhuyzen concluded that the less refractive rings are composed of less hydrated amylose. The hydration of the amylose may depend upon the physiological behavior of the plastid in which it is laid down. The latter is a function, no doubt, of illumination and transpiration. When these are uniform, ring formation does not occur, and the needles grow uniformly from center to periphery as in the instance of his experiment.

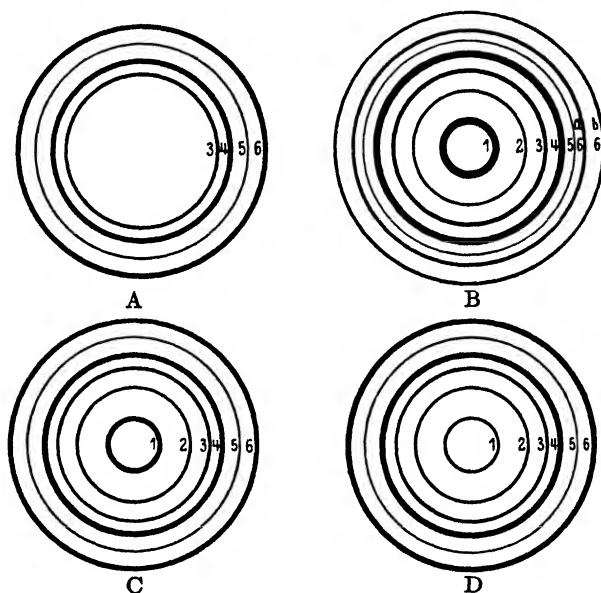


Fig. 11. Types of X-ray spectra of natural (A, B, C) starch granules after Katz and Itallie (17).

A. Spectrum B. Spectrum with heavy 1-Ring
C. Spectrum with light 1-Ring D. Spectrum with light 1-Ring.

This regular arrangement of microcrystals imparts to raw starch the property of yielding an x-ray spectrum with several sharp, well-defined concentric rings. Wheat starch, like rye, oats, rice and corn, yielded what Katz and Itallie¹⁷ describe as the "A. spectrum" shown in Fig. 11. Potato and chestnut starches, for example, gave a "B spectrum," Manihot an intermediate or "C" spectrum." In the A spectrum, the No. 1 and No. 2 rings were missing, and the No. 6 ring was simple, whereas in the B spectrum a heavy No. 1 ring, a weak No. 2 ring, and a double No. 6 ring could

¹⁷ *Z. physik. Chem.*, 150, 90 (1930).

be discerned. In the intermediate or C spectra, the No. 1 ring was either weak or medium in strength, and the No. 6 ring was simple.

When suspensions of starch granules were heated, the original x-ray spectrum gave place to a V-spectrum (V for "Verkleisterung"), which was more or less common to all starches regardless of the original spectrum of the raw starch. In the V spectrum the No. 1 ring, if originally present, had disappeared; also the No. 3 ring. The heavy No. 4 ring became light or nebulous; the No. 5 ring, on the other hand, became heaviest; and the No. 6 ring, like the No. 4 ring, became light.

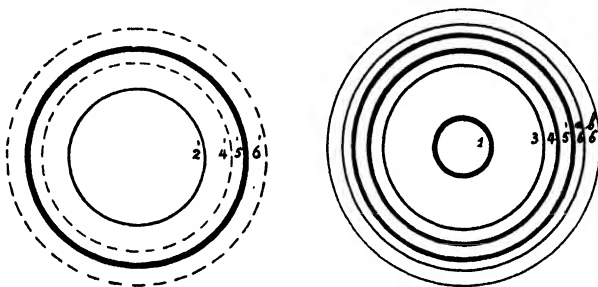


FIG. 12. X-ray spectra of gelatinized starch (left) described by Katz (17) as the V-spectrum, and retrograded wheat starch (right).

When such pastes were allowed to age, they underwent a retrogradation, which was accompanied by a change in the x-ray spectrum, as shown in Fig. 12. Katz and Itallie¹⁷ found the same x-ray spectrum for both the retrograded amylose and amylopectin of starch, with characteristics common to the B spectrum. Evidently the pasting of starch disturbed the regularity of arrangement of the molecules in the micro-crystals, with the loss of certain spectral lines. With the lapse of time in aging, these pastes tended to regain a more regular arrangement, probably with the squeezing out of water or dilute starch solution as is frequent in the process of syneresis of such systems. This partial dehydration brought the starch molecules or micelles into closer proximity, with a consequent increase in the regularity of the structure that resulted in a new spectrum with additional or sharper lines.

Sharpening of these x-ray spectrographic lines resulted when a small quantity of water was added to starch in Bear's¹⁸ researches at Iowa State College. This suggested that the forces exerted in holding the glucose units in the crystalline pattern may actually be exerted through the absorbed water rather than through the groups of the anhydrous starch

¹⁸ Private communication (1941).

itself. Bear's excellent x-ray spectrographic plates tend to support the earlier observations of Katz, but may make possible more extensive interpretation and mathematical analysis.

At the time of the publication of Naegeli's¹⁹ treatise on starch, the idea was current that the starch granules consisted of more than one substance. Meyer²⁰ proposed the terms alpha-amylose and beta-amylose for the two prominent materials; Maquenne and Roux²¹ called them amylopectin and amylose respectively. Samec and co-workers prefer the terms amylo amylose and erythro amylose for the two starch fractions obtained by his process of electrophoresis, with the latter migrating to the anode. The significance of these terms is determined in part by the methods actually employed in fractionating the substances.

A fairly considerable number of methods have been proposed for separating and recovering the materials in the starch granule. Tanret²² used separation by sedimentation, whereas Sherman and Baker²³ employed a centrifugal method, which separated the paste made with dilute NaCl into a heavier, very viscous, opalescent layer containing more than 90 per cent of the material presumed to be the α -amylose of Meyer, or the amylopectin of Maquenne, and a lighter, limpid solution containing more than 90 per cent of the more soluble β -amylose. The two fractions behaved quite differently when various amylolytic enzymes were added.

Obermeyer²⁴ applied this method to wheat starch and obtained about 70 per cent of the dry substance in the "amylopectin" fraction and only 30 per cent in the "amylose" fraction. This is about the reverse of the estimates of Maquenne and others, and suggests that the fractionation was not complete. Obermeyer's "amylose" fraction contained only about half as much phosphorus as the "amylopectin," to be sure, but even this difference was less than that of the preparations made by certain other methods, notably electrophoresis, as will appear later.

Various solvents have also been tried in the effort to find one which would dissolve and remove one of the amyloses selectively. Solutions of calcium and barium hydroxide (10 per cent) followed by water, alcohol-water solutions of ammonium thiocyanate, and acidified methyl alcohol were employed to extract "amylose;" but none has been generally accepted as quantitative procedures. Likewise, selective adsorption as upon freshly precipitated alumina hydroxide was tried in an effort to remove amylopectin from a paste, but the method has not been adopted for general use.

¹⁹ "Die Stärkekörner," Zurich (1858).

²⁰ "Untersuchungen über die Stärkekörner," Jena (1895).

²¹ *Bull. soc. chim.*, 33, 723 (1905).

²² *Bull. soc. chim.*, 17, 83 (1915).

²³ *J. Am. Chem. Soc.*, 38, 1885 (1916).

²⁴ "Thermal fractionation of starch." Thesis filed in the Library of the University of Minnesota (1934).

Gatin-Gruzewska²⁵ treated a starch paste with alkali solution at 60°, added one-third of its volume of strong alcohol, and the "amylopectin" was precipitated. This was neutralized, and purified by dialysis or decantation. Amylose could be recovered from the mother liquor. In another experiment 1 per cent NaOH solution was used in treating the starch suspension. The amylose was presumed to burst out of the swollen granules. Evidently Gatin-Gruzewska presumed that the starch "hull" was amylopectin exclusively, and any procedure which separated the membrane or hull from the interior content of the granule, in effect, was separating the amylopectin from the amylose.

Amylopectin (I) was distinguished structurally from amylose (II) by Ling and Nanji²⁶ to the extent that II was depicted as an α -hexa-amylose, while I was diagrammed as an $\alpha\beta$ -hexa-amylose, *i.e.*, with two β linkages to four α linkages. In addition, the amylopectin (I) was stated to be a phosphoric acid ester of the hexa-amylose, with two phosphoric acid groups to the six hexose residues.

Later they²⁷ used barley diastase to convert (*i.e.*, hydrolyze) the "amylose" of wheat starch, and on this basis of classification they found 66.3 per cent of amylose and 33.7 per cent of amylopectin. They agree that the values are merely relative, however, and that the amylopectin content is doubtless smaller, since the estimate given must be corrected by deducting the percentage of "hemicelluloses" present. They²⁸ gave the name "amylo-hemicellulose" to the latter, and decided that it was not hydrolyzed by barley diastase. Moreover, it appeared to be a calcium-magnesium or iron salt of a silicic ester of the polysaccharide. As determined by recovering it as the flocculate form in a digestate of a starch paste treated with barley extract, a yield of 7-8 per cent was obtained from wheat starch. Another somewhat different method gave 10 per cent of the amylo-hemicellulose in wheat starch, more in rice, and less in barley.

Ling and Nanji also used a thermal fractionation which involved freezing a 5 per cent starch paste, that was slowly thawed, and then extracted with water to remove the more soluble amylose. This method was employed by Hirst, Plant and Wilkinson.²⁹ A 5 per cent paste was made by heating the starch suspension for 30 minutes at 90-100°, stirred at 0° until it solidified, and left overnight at -10° to -15°. The white fibrous mass was extracted several times with H₂O at 60°, the extract concentrated at 50° under diminished pressure, and ethyl alcohol added. The precipitated amylose, when thus prepared from potato starch, gave a deep

²⁵ *Compt. rend.*, 146, 540 (1908).

²⁶ *J. Chem. Soc.*, 123, 2666 (1923).

²⁷ *Ibid.*, 127, 629 (1925).

²⁸ *Ibid.*, 127, 652 (1925).

²⁹ *J. Chem. Soc.*, 2375 (1932).

blue color with iodine, had a specific rotatory power in H_2O when freshly prepared, of $(\alpha)_D^{20} = +190^\circ$, while in 5 per cent NaOH $(\alpha)_D^{20} = +151$. The phosphorus content, calculated as P_2O_5 , was 0.20 per cent. The material which remained after long-continued extraction of the fibrous mass with warm water, and considered to be "amylopectin," could be converted into a paste with H_2O at 90° , and gave a purple color with iodine in a concentrated paste; but the color was blue and indistinguishable from amylose when the paste was diluted; $(\alpha)_D^{20}$ in 5 per cent $\text{NaOH} = +151^\circ$, and its phosphorus content (calculated as P_2O_5) was 0.20 per cent. Obermeyer²⁴ applied this procedure to wheat, corn, and potato starch. The yield of amylose that could be extracted from the fibrous mass was small, being as low as 3.2 per cent with one of the potato starch preparations, whereas Hirst *et al.* obtained 17 per cent. The wheat starch preparations yielded an average of 2.8 per cent, the corn starch preparations 4.5 per cent of "amylose."

Since Maquenne and others had previously indicated that most of the starch granule was amylose ("β-amylose"), it seems evident that these procedures employed by Hirst *et al.*, and Obermeyer, did not effect a good fractionation. Probably much of the amylose had been retrograded to a relatively insoluble form by the freezing treatment, and this appeared in the "amylopectin" fraction. This is suggested further by the lack of difference in the phosphorus content of the two fractions reported by Hirst *et al.* It also receives some support in the observations made by Woodruff³⁰ with the Spierer lens. Wheat amylose, previously separated from the amylopectin by electrophoretic deposition of the latter, was pasted with hot water, and then frozen. Not only did fibrous strands become apparent on subsequent thawing of the preparation, but their presence was confirmed by characteristic striations disclosed by the Spierer lens. Moreover, in like manipulations of ordinary starch pastes, the retrograded material which appeared upon freezing represented a large proportion of the dry substance of the original preparation. It was not unlike a wad of wetted cotton in texture, although, to be sure, the individual "fibers" do not possess the tensile strength of cellulose fibers.

Samec³¹ credits Gatin-Gruzewska³² with the discovery of the anodic migration of the amylopectin fraction of starch, although it appears that Coehn³³ had observed that starch in solution had a negative charge, and Bottazzi and Victorow³⁴ also effected the transport of amylopectin to the anode. The brilliant application of this technique was developed in

³⁰ *Ind. Eng. Chem.*, 30, 1409 (1938).

³¹ *Trans. Faraday Soc.*, 31, 395 (1935).

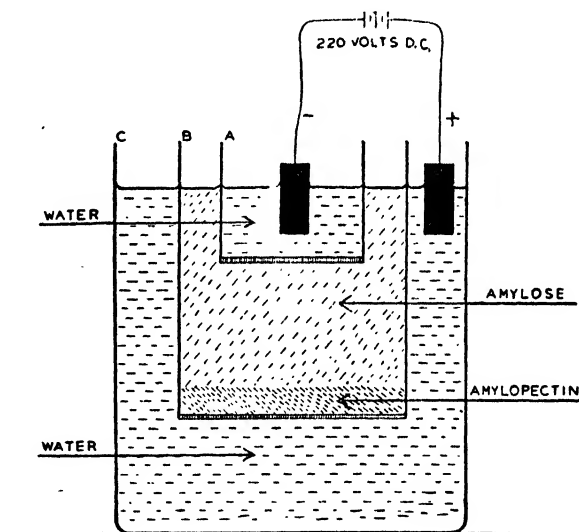
³² *J. physiol. path. gen.*, 14, 7 (1912).

³³ *Z. Elektrochem.*, 4, 68 (1897).

³⁴ *Reale accad. Lincei. Atti.*, 19, 7 (1910).

Samec's laboratory, and his findings are recorded in an extensive series of papers. The procedure was described by Samec and Haerdtl,³⁵ and was later elaborated upon by Taylor and Iddles.³⁶ A vertical cell of three chambers separated by two collodion membranes was employed. The cathodic electrode was located in the upper and smallest chamber, the anodic electrode in the lower and largest chamber. Amylopectin ("α-amylase"), being negatively charged, traveled downward with the current and accumulated on the lower membrane, whence it could be recovered. The construction of the cell was further modified by Stamberg and Bailey,³ who increased greatly the surface of the membranes. Their design is shown diagrammatically in Fig. 13.

FIG. 13. Diagram of electro dialyzing apparatus used by Stamberg and Bailey³ in the recovery of "amylopectin."



Amylopectin was recovered from wheat starch by Samec, Minaeff and Ronzin³⁷ by electro dialysis of a starch solution heated for 30 minutes at 120° in the amount of 63 per cent and on heating for 60 minutes Samec and Haerdtl³⁵ recovered 59.6 per cent.

Before describing the preparations recovered by these electrophoretic techniques, mention should be made of the ingenious practice, introduced by Taylor and Beckmann,³⁸ of grinding the starch granules for protracted periods in a ball mill. This was later elaborated upon by Taylor and Salzmann³⁹ to include a test of the completeness of the mechanical rup-

³⁵ *Kolloidchem. Beihefte*, 12, 281 (1920).

³⁶ *Ind. Eng. Chem.*, 18, 713 (1926).

³⁷ *Kolloid-Beihefte*, 19, 203 (1924).

³⁸ *J. Am. Chem. Soc.*, 51, 294 (1929).

³⁹ *J. Am. Chem. Soc.*, 55, 264 (1933).

turing of the starch granules in terms of "alkali lability." This test made it possible to graduate the grinding operation by applying such tests periodically. The procedure was studied more elaborately by Taylor and Keresztesy.⁴⁰ Stamberg and Bailey³ found a rod mill of their own construction to be decidedly superior to the conventional ball mill, both in terms of rapidity and in uniformity of treatment. They standardized the grinding of wheat starch at 84 hours of continuous treatment. In this way the starch granules were mechanically disintegrated before pasting, thus facilitating the recovery of fractions free from intact granules.

Early in Samec's studies attention was called to the tendency of the phosphorus-containing groups to accumulate in the amylopectin fraction which migrated toward the anode under electrophoresis. In fact, after two decades or more of researches, Samec³¹ contended that "starch owes its electric charge principally to phosphoric acid," although he concedes, "In some starches also fatty acids and carboxyl groups contribute to the charge." Samec's⁴¹ idea of the linkage of the phosphoric acid was not identical with Irvine's, however, since he proposed that it might be linked with the nitrogenous substance known to be present. This is supported by the views of Koets,⁴² who proposed that amylopectin is a complex coacervate of amylophosphoric acid and a nitrogenous substance, probably of protein character. Thus when the nitrogen content was diminished by manipulation, the surplus electric charge decreased.

Stamberg and Bailey³ found that most of the phosphorus of the wheat starch granule appeared in the amylopectin fraction that was recovered by their electrophoretic method, the average of 10 preparations being 86 per cent recovery. All the wheat amylose preparations were very low in their content of phosphorus, none exceeding 0.007 per cent, and some being as low as 0.001 per cent. The total phosphorus content of the original wheat starches averaged 0.062 per cent with a range from 0.050 to 0.073 per cent. The nitrogen content of the same starches averaged 0.047 per cent, and ranged from 0.035 to 0.056 per cent. An average of 15.0 per cent of amylopectin was recovered from the starch pastes by fractionation electrophoretically.

Mention should be made here of Mangel's¹³ observation of substantial differences in the total phosphorus content of starches separated from wheats grown in different crop seasons. The *vulgare* wheat starches of the 1928 crop averaged 0.056 per cent P, those of the 1932 crop 0.072 per cent. Incidentally, 1928 was a season of normal rainfall, while in 1932 there was a lighter precipitation. The nitrogen content of the starch

⁴⁰ *Ind. Eng. Chem.*, **28**, 502 (1936).

⁴¹ *Z. ges. Getreide-, Mühlen- u. Bäckereiw.*, **21**, 111 (1934).

⁴² *Koninkl. Akad. Wetenschappen Amsterdam*, **38**, 3 (1935).

samples was much more uniform than the phosphorus content, and ranged around 0.045 per cent.

Kniaginichev and Gorelkina⁴³ also observed rather substantial variations in the total phosphorus content of starch granules from several genetic species of the genus *Triticum*. Two samples prepared from *T. durum* contained 0.103 and 0.104 per cent respectively, while two *vulgare* varieties contained only 0.061 and 0.055 per cent. When *Lutescens* 062 wheat was grown on a control or unfertilized plot, the phosphorus content of its starch was 0.096 per cent, but that from a plot receiving phosphate fertilizer was 0.128 per cent. Another variety, *Hordeiforme* 010, did not respond in terms of phosphorus content of the starch when it was fertilized with phosphates, however, so the result of such treatments was not consistent.

Most of the phosphoric acid of wheat starch was split off by treatment with a suitable phosphatase (from kidney) in the experiments of Waldschmidt-Leitz, Samec and Mayer.⁴⁴

Taylor and Walton⁴⁵ fractionated the " α -amylose" from wheat starch and obtained yields of 23½ per cent after permitting the anodic deposition to continue for as long as 46 days. While no ether-soluble material could be recovered by direct extraction of the α -amylose preparation, they obtained 1.18 per cent after hydrolysis, as contrasted with 0.58 per cent in the original wheat starch. Thus the fatty material appeared largely in the α -amylose fraction. The phosphorus content of two α -amylose preparations was 0.265 and 0.353 per cent respectively, while a β -amylose that was recovered contained only 0.041 per cent. Despite these differences in phosphorus content, Taylor, in conversations and correspondence with the writer, stated that he was of the opinion that the phosphorus was not chemically combined in the α -amylose molecule. This view may have been conditioned in part by the earlier observation of Taylor and Iddles³⁶ that the phosphorus content of β -amylose prepared from potato starch was actually higher than the phosphorus content of the α -amylose from the same material. It was also the consequence of an observation that the phosphorus-containing groups could be separated from the α -amylose without altering the latter; but the details of this experiment apparently were not published by him before his death. In the same exchanges he also stated that not only were the fatty acids a part of the α -amylose molecule, but that he was already beginning to arrive at some indications of the order or sequence in which they were linked along the chain of glucose units.

⁴³ *Compt. rend. acad. sci. U.S.S.R.*, 19, 117 (1938).

⁴⁴ *Z. Physiol. Chem.*, 250, 192 (1937).

⁴⁵ *J. Am. Chem. Soc.*, 51, 3431 (1929).

As a matter of fact, Taylor's interest in the fatty acids of starch was of long standing. As early as 1920 Taylor and Nelson⁴⁶ had observed the presence of substantial proportions of the higher fatty acids in the refinery mud appearing in the processing of corn starch. After further studies of the fatty acids of corn starch, Taylor and Lehrmann⁴⁷ turned their attention to wheat starch and reported that 35 per cent of palmitic, 41 per cent of oleic, and 24 per cent of linoleic acids comprised the mixture of fatty acids from that source.

Schoch⁴⁸ contended that these fatty acids are not chemically combined with any fraction of the starch, but are merely adsorbed within the granule. While the fatty acids cannot be extracted from the cereal starches by such solvents as ethyl ether or carbon tetrachloride, they are completely removed by certain hydrophilic fat solvents, preferably 80 per cent dioxan—20 per cent water, or 85 per cent methanol—15 per cent water, or by "Methyl Cellosolve." The fatty acids thus extracted from cornstarch ranged in acid number from 134 to 153; the fatty material from wheat starch appeared to be a phospholipid, containing practically all the phosphorus present in the original raw starch.

Removal of fatty material did not alter the alkali lability of the starch, as measured by Schoch's method.⁴⁹ The granule structure remained intact, though the defatted starches gave clearer pastes of greater gelling power than the native starches. By treating potato starch or defatted cornstarch with a solution of oleic acid in methanol or "Methyl Cellosolve," as much as 2 per cent of fatty acid could be re-introduced into the granule, and this added fat could be removed only with hydrophilic solvents.

When a cornstarch paste was solubilized by prolonged boiling or by autoclaving and then treated with butanol, Schoch⁵⁰ obtained a microscopic semi-crystalline precipitate. This was best isolated with the supercentrifuge, and could be purified by solution in hot water and reprecipitation with butanol, consistently amounting to 21-23 per cent of the original raw starch. When dehydrated with ethanol, this amylose fraction became completely insoluble in hot or cold water. Its most distinctive chemical characteristic was an alkali lability considerably higher than the raw cornstarch. In contrast, the starch fraction which was not precipitated by butanol was relatively soluble in water, with little tendency to retrograde. Its alkali lability was correspondingly lower than that of the raw starch.

⁴⁶ *J. Am. Chem. Soc.*, **42**, 1726 (1920).

⁴⁷ *J. Am. Chem. Soc.*, **52**, 808 (1930).

⁴⁸ *J. Am. Chem. Soc.*, **60**, 2824 (1938).

⁴⁹ *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).

⁵⁰ *Cereal Chem.*, **18**, 121 (1941).

Although their analyses were restricted to corn starch, the observations of Evans and Briggs⁵¹ are worthy of note here. They found corn starch obtained from corn kernels where the embryos were removed before milling to contain 0.64 per cent of fatty material (dry basis), which was identical with factory starch in that particular. The fatty acids constituted about 94 per cent of the lipids recovered after acid hydrolysis of the starch, and of these fatty acids, oleic acid represented 37.7 per cent, linolic acid 31.1 per cent, and linolenic acid 1.2 per cent. Thus the unsaturated fatty acids constituted 70 per cent of the total fatty acids. The mean molecular weight of the 30 per cent of saturated fatty acids was 269.0.

Extraction which removed 95 per cent of the lipids lowered the phosphorus content of the starch from the original content of 0.016 to 0.010 per cent. Accordingly, only a small portion of the phosphorus present can be assumed to be in phosphatide combination.

Although the researches upon the amyloses by Meyer *et al.*⁵² were conducted chiefly with corn (maize) starch, certain of their general conclusions probably have more general application. Corn starch appeared to be composed of two polysaccharides, (1) amylose, representing 10-20 per cent, with unbranched chains, and having a molecular weight of 10,000 to 60,000; (2) amylopectin, representing 80-90 per cent, with branched chains, and having a molecular weight of 50,000 to 1,000,000.

Amylose could be extracted with hot water (80°) and was soluble in warm formamide, 33 per cent chloral hydrate, hydrazine hydrate, ethylene diamine hydrate, LiBr, ZnCl₂; has a specific rotatory power of $(\alpha)_D = +195^\circ$ to $+197^\circ$, and when dried *in vacuo*, a molecular composition of C₆H₁₀O₅. It has a terminal group for every 30 glucose residues.

Amylopectin was recovered from the residue of the treatment of starch with warm (80°) water. A reticular structure involving branched chains is depicted. When it was treated with β -amylase, hydrolysis equivalent to 54 per cent was effected in 120 minutes, and then the residual dextrin or erythrogranulose stage was reached. With amylose as the substrate, hydrolysis in excess of 73 per cent was evidently attained.

In a later paper Meyer, Wertheim and Bernfeld⁵³ speculate further concerning the constitution of the residual erythrogranulose which remains after the action of β -amylase on amylopectin. Several branched-chain formulas are presented which might represent polysaccharides wholly resistant to the action of this enzyme.

⁵¹ *Cereal Chem.*, **18**, 443 (1941).

⁵² *Helv. Chim. Acta*, **23**, 845; 854; 865; 875; 885; 890; 1465 (1940).

⁵³ *Ibid.*, **24**, 212 (1941).

To amylopectin Seck and Fischer⁵⁴ assigned the role of the structural element in starch gels.

Janicki⁵⁵ also was in disagreement with Samec's views and submitted that the major portion of the phosphorus, namely 92-98 per cent, is adsorbed on starch as colloidal phosphates of Ca, Mg, Na, K, and Fe, the remainder being in the form of an ester.

It has long been known that the principal constituents of the starch granule are condensation products of glucose. As early as 1815 Kirchhoff⁵⁶ had effected the separation of a crystalline, saccharine substance from starch, and associated it with the early stages of the alcoholic fermentation of starch. By gradual stages an extensive literature developed which disclosed that maltose appeared in consequence of the action of amylases ("diastase") upon starch, and that maltose, in turn, is a glucose- α -glucoside. It was also deemed probable that the linkages between the maltose units are also of the α -glucosidic type (more correctly, α -malto-sidic), which implies a uniform *type* of linkage through the entire amylose molecule, assuming that maltose units constitute the entire molecule.

Selective adsorption of β -amylose was effected by Pacsu and Mullen⁵⁷ on activated carbon, fuller's earth and Brockman alumina, whereas the α -amylose remained in solution. They obtained better results by the use of cellulose (cotton). The absorbate was broken with hot water to yield a clear solution of pure β -amylose. While the latter could be precipitated with alcohol, the preparation so obtained became insoluble in water when dried in a desiccator. Also the aqueous solution retrograded rapidly, giving a turbid solution in which granules of retrograded β -amylose appeared that were insoluble in cold water. The presence of pyridine in the aqueous solution prevented this retrogradation.

α -Amylose was obtained by repeatedly passing the mixture of amyloses through cotton, and then precipitating the α -amylose from the solution with alcohol. It had a specific rotatory power of $(\alpha)_D^{20} = 145^\circ$, as did also the β -amylose. The α -amylose preparation contained 0.020 per cent of phosphorus, but the β -amylose was free from that element. The β -amylose gave a deep blue color with iodine, whereas the α -amylose showed a purple color.

This assumption of α -glucosidic linkages received support from certain observations, including the fact that no glucosans comprising more than two glucose units were derived by simple fission of amylose that have been identified as having β -glucosidic linkages; the x-ray spectra were generally interpreted as suggesting a uniform type of linkage; also

⁵⁴ *Kolloid-Z.*, **93**, 207 (1940).

⁵⁵ *Roczniki Chem.*, **12**, 402 (1932).

⁵⁶ *Schweigger's J. chem. u. physik.*, **14**, 389 (1815).

⁵⁷ *J. Am. Chem. Soc.*, **63**, 1168 (1941).

the general behavior of amyloses on hydrolysis catalyzed by amylases or diastases was accepted as supporting this assumption. The models presented by Haworth^{58, 59} are typical of much of the reasoning and speculation in that field during the past decade. It was conceded, however, that the projection of the poly- α -maltosidic molecule into three-dimensional space introduced various possibilities. Thus, it was suggested that we need not assume the amylose molecule to be "straight," i.e., its glucopyranose rings need not lie along a straight line, or even in the same general plane. It might loop around a rough circle, or even more probably, it might assume a spiral or helical pattern.

Another, and even simpler structure was suggested by Irvine,⁶⁰ in which six glucose units are joined in a series of α -glucosidic linkages into a closed ring, conveniently described as an anhydro-hexaglucose. Other variations were sounded on this type of structure, notably by Pringsheim. As time went on, it appeared to be inadequate from several points of view, notably the small size of the molecule, and the possession of properties by amyloses which could not be explained either from the configuration or dimensions of the Irvine molecule.

Concurrently with these studies, the application of x-ray methods to the measurements of crystal structure had disclosed the space occupied by C, H, and O atoms, and the possibilities of building strainless molecular models. Such calculations combined with direct measurements of the diameter of gluco-pyranose rings resulted in an estimate of 5.05 Å for the diameter of such rings in the plane bounded by the No. 3 and No. 5 carbon atoms and not including the side chains. The author has calculated that if the individual amylose molecules lay uniformly parallel in the granule and were essentially straight (which they probably are not, as indicated in the preceding paragraph), a line projected across a wheat starch granule of the largest size, say 37.5 μ , will pass through 75,000 amylose molecules. This estimate, while based upon some questionable assumptions, is convenient in graphically depicting the compactness of such a granule.

A third type of structure was proposed by various researchers, notably Staudinger and Husemann.⁶¹ They concluded that the simple Haworth model could not be fitted to certain observed physical properties of starch. A molecule with a series of main chains such as the Haworth school had proposed, but with branched chains, some of which are linked through the No. 6 carbon or primary alcoholic group of the glucose units involved, some through the No. 3 carbon, would conform better with the observed data. This would introduce the equivalent of gentiobiose units into the

⁵⁸ "The Constitution of Sugars," pp. 50-52, New York and London (1929).

⁵⁹ "Rapports sur les hydrates de carbone (glucides)," Union Inter. de Chimie, Paris (1930).

⁶⁰ *Chem. Rev.*, **4**, 208 (1927).

⁶¹ *Ann.*, **527**, 195 (1937).

molecule, in addition to the predominant maltose units. The presence of such gentiobiose linkages received support from the earlier work of Berlin,⁶² who isolated gentiobiose from certain residues ("hydrol"), remaining from the manufacture of glucose from hydrolyzed corn starch. It should be indicated, however, that the presence of gentiobiose in this hydrol is not definite evidence of the occurrence of gentiobiose units in the starch molecule. It is conceivable that it was a product of synthesis from the glucose present in the acid hydrolyzate, appearing as did Fischer's isomaltose.

It should also be noted that the Staudinger model as depicted in Fig. 14 would yield on methylation and subsequent hydrolysis, not only 2, 3, 4, 6-tetramethylglucose, and 2, 3, 6-trimethylglucose, but a 2, 6- and a 2, 3-dimethylglucose as well.

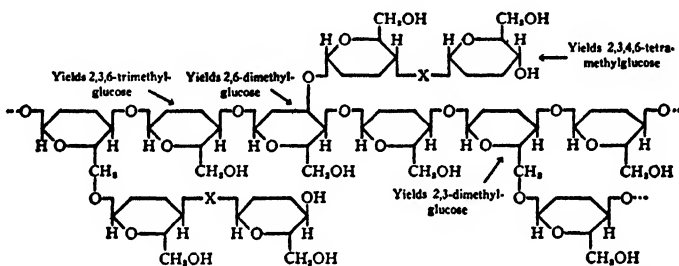


FIG. 14. Staudinger's⁶¹ diagram of the starch molecule showing gentiobiose linkage. (X = 20 glucose units)

Myrback and Ahlborg⁶³ apparently accept the same general branched-chain concept, and Myrback⁶⁴ further suggested that the incomplete hydrolysis of starch effected by β -amylase may be the consequence, in part, of these branched chains.

Recently Hudson⁶⁵ stressed the fact that the production of 2, 3, 6-trimethyl glucose on the hydrolysis of methylated amylose means that positions 4 and 5 are occupied by linkages in the amylose molecule, but did not establish the kind of linkage. His recovery of *d*-erythronic acid on the appropriate chemical manipulation of starch involved a yield far below the theoretical if maltose alone constituted the units of starch. He proposed the occurrence in the starch molecule of acetal linkages involving the No. 4 and 5 carbons of certain of the gluco-pyranose structures. The Schardinger dextrans recovered by enzymic or biological treatment of starch suggests that these dextrans are components or units of the starch molecule.

⁶² *J. Am. Chem. Soc.*, **48**, 2627 (1926).

⁶³ *Svensk. Kem. Tid.*, **49**, 216 (1937).

⁶⁴ *Current Sci.*, **6**, 47 (1937).

⁶⁵ Paper presented before the 26th annual meeting of the Amer. Ass'n. Cereal Chemists, New York, 1940.

In a discussion of these possibilities Pacsu proposed further that the 4, 5 acetal linkage of one gluco-pyranose unit may be joined to the No. 1 carbon of another gluco-pyranose unit, resulting in cross linkages of a chemical nature different from the type proposed by Staudinger *et al.*⁶¹

A branched-chain structure is depicted by Meyer⁶⁰ for amylopectin, to which he assigns a molecular weight in excess of 300,000. He contends that the amylopectin of cereal starches does not contain any chemically bound phosphoric acid, since preparations which contain phosphorus because of admixed phosphatides can be freed of phosphorus by treatment with appropriate solvents. Amyloses, on the other hand, are represented by Meyer as unbranched, and the molecules are smaller than those of amylopectin, with a molecular weight between 10,000, and 100,000. Amylose as thus defined is completely hydrolyzed by β -amylose, while amylopectin is broken down to a residual dextrin of high molecular weight.

Molecular Weight of Starch and the Amyloses

There is a wide diversity of opinion expressed in the literature concerning the molecular weight of "starch" and of the amyloses. Part of the difficulty doubtless arises from a lack of agreement as to the use of the term *molecule*. Also it seems probable that associated molecules or aggregates are not always clearly distinguishable from true molecules, particularly when physical methods are employed in the measurement of aggregate dimensions.

The Haworth school based their estimates upon the purely chemical approach afforded through the application of the Purdy methylation techniques as improved by Haworth and by Irvine. Completely methylated amyloses were hydrolyzed by Hirst, Plant and Wilkinson⁶⁷ in the Birmingham laboratory, and it was estimated from the percentage of tetramethyl glucose appearing on hydrolysis of the methylated amylose that the average chain length of both amylose and amylopectin was 24 glucose units. This would be equivalent to a molecular weight in the range of 4,000. The mercaptalation method, as applied by Wolfrom, Myers and Lassetre,⁶⁸ indicated an initial average degree of polymerization of potato starch of 20 ± 4 units.

Kratky and Mark⁶⁹ suggest two possibilities: (1) that the end-group analysis, as developed and applied by Haworth and associates, discloses the principal or primary valences in the molecule, whereas osmotic methods involve micelles resulting from the operation of secondary valence forces; (2) that osmotic methods disclose the correct molecular weight and

⁶⁶ "Advances in Colloid Chemistry," edited by E. O. Kraemer, Vol. I, p. 143 (1942).

⁶⁷ *J. Chem. Soc.*, 2375 (1932).

⁶⁸ *J. Am. Chem. Soc.*, 61, 2172 (1939).

⁶⁹ *Fortschr. Chem. org. Naturstoffe*, 1, 255 (1938).

the apparent number of end groups is contingent upon the branching of the molecule such as was proposed by Staudinger and Husemann.⁶¹

Hess and Lung,⁷⁰ using the end-group test, found the chain length of starch to be equivalent to about 50 glucose units. Based upon the viscosity, using Staudinger's equation, the number of glucose units would be equivalent to from 1600 to 3100 units, however. They challenge the adequacy of the Haworth tetramethyl glucose fractionation on the one hand, and further suggest that any such manipulation as methylation and subsequent hydrolysis is influenced largely by the previous treatment of the starch preparations used in such studies.

Oxidation of starch with periodic acid as practised by Caldwell and Hixon⁷¹ led them to the conclusion that the chain length is much greater than 25 glucose units.

Staudinger and Husemann's⁷² estimates of the degree of polymerization of wheat starch, based upon viscosity methods, ranged from 600 to about 1800 glucose units. They further propose that the contention as to the distinction between true molecules and molecular aggregates held together by secondary valence forces, may be disposed of by converting the assumed "molecule" into a derivative, and noting the molecular size of the derivative. If, as in the instance of starch acetates, there is no change in the degree of polymerization, and from these acetates the original starch could be regenerated, this, in their judgment, tends to establish that the original starch was comprised of actual molecules, whose size or degree of polymerization had been disclosed.

Richardson, Higginbotham and Farrow⁷³ based their calculations of the chain length of the starch molecule upon the copper number. Assuming that each chain molecule terminates at one end in a reducing group, and that each of these groups reduces as much copper as does the reducing group in maltose, the average chain length of starches is 460-1470 glucose units.

Thomas⁷⁴ estimated from the phosphorus content of purified potato starch preparations that the molecular weight was in the range of 200,000.

Rodewald⁷⁵ based an estimate upon the heat of wetting in its relation to surface area of starch molecules and arrived at values equivalent to molecular weights from 17,000 to 103,000. He also⁷⁶ converted starch into a starch-iodide preparation and determined its osmotic pressure from

⁷⁰ *Ber.*, 71, 815 (1938).

⁷¹ *J. Biol. Chem.*, 123, 595 (1938).

⁷² *Ber.*, 71, 1057 (1938).

⁷³ *J. Textile Inst.*, 27, 131 (1936).

⁷⁴ *Biochem. Bull.*, 3, 403 (1913-1914).

⁷⁵ *Z. physik. Chem.*, 33, 593 (1900).

⁷⁶ *Z. physik. Chem.*, 33, 579 (1900).

which he calculated the molecular weight of the starch iodide to be 36,700 to 39,700.

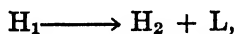
Oeholm ⁷⁷ employed a refractometric method and calculated the molecular weight of starch in the powder form to be 10,000.

Brown and Harris,⁷⁸ using the cryoscopic method of Raolt, calculated the molecular weight to be 32,400.

Walden ⁷⁹ determined the freezing point depression of purified starch in formamide solution and found the average molecular weight to be 645.

Lamm ⁸⁰ based estimates upon the sedimentation velocity in the ultracentrifuge which indicated 5,550 glucose units in the starch molecule.

Using a Beams ultracentrifuge, Beckman and Landis ⁸¹ determined the sedimentation constant of β -amylose from corn starch and found it to be of the order of 1.3 to 12×10^{-13} . This β -amylose was separated from the α -amylose by the electrophoretic procedure of Taylor and Iddles.⁸⁶ It was heterogeneous with respect to particle size, the molecular weights ranging from 17,000 to 225,000. About one-half of the material had a molecular weight in the narrower range of from 31,000 to 60,000. In a lecture on his researches in this field presented before the American Association of Cereal Chemists in May, 1940, Dr. Beckman observed that the sequence in the degradation of starch is indicated by ultracentrifugal methods to result first in two components thus:



in which the component L has a molecular weight of 17,500. Thereafter the progress of degradation becomes:



the molecular weight of L being about the same in all cases and representing a fundamental unit of the starch molecule.

Other researches in Beckmann's laboratory have indicated molecular weights of the same general order of magnitude for potato starch.

Samec and Mayer,⁸² basing their estimates upon osmotic pressure methods, reported molecular weights of potato starch amylose and amylopectin as 80,000 and 113,000 respectively. Later Samec ⁸¹ estimated that the osmotically active micelle of amylophosphoric acid prepared from potato starch would be represented by the formula $(C_6H_{10}O_5)_{1240}$

⁷⁷ Medd. K. Vetenskapakade. Nobelinst., 2, 1 (1913).

⁷⁸ J. Chem. Soc., 55, 462 (1889).

⁷⁹ Bull. Acad. imp. sci. St. Petersburg, 5, 1055 (1911).

⁸⁰ Kolloid-Z., 69, 44 (1934).

⁸¹ J. Am. Chem. Soc., 64, 1495 (1939).

⁸² Kolloidchem. Beihefte 13, 272 (1921).

(H_2PO_3)₅. Diffusion methods did not yield useful results in these measurements.

Bergmann and Knehe⁸³ reported the molecular weight of starch amyloseacetate (in phenolic solution) in the range of 289-326, while Bergmann, Knehe and Lippmann⁸⁴ obtained values in the same general range in glacial acetic acid, and for amylopectin acetate in the same two reagents. The conditions of acetylation (alkaline) were such as to suggest that cleavage of the amylopectin and amylose had occurred before the molecular-weight determinations were applied.

In an excellent summary of these conflicting views concerning the dimensions and configurations of the starch (amylose) molecule Armstrong⁸⁵ mentions the calculation of Richardson, Higginbotham and Farrow,⁸⁶ that if hydrolysis of only 3 per cent of the linkages occurred in a chain of 10,000 units, the latter becomes 301 chains of average length of 33.2 units, which is in the range of the Haworth estimate. Contrary to Haworth and Percival,⁸⁷ Richardson *et al.* found unmodified starches to have definite reducing powers ranging from 2.8 to 8.9, in which maltose = 2055, and corresponding to chain lengths of 1470 to 460 glucose units. These are of the order of magnitude of 20-50 times those obtained by Haworth's methylation method.

Staudinger and Husemann⁷² contend that the physical properties of starch suggest the existence of macro-molecules as distinct from molecular aggregates. In a formamide solution the osmotic pressure measurements indicate a molecular weight equivalent to 1770 glucose units.

The Starch Content of Wheat and Wheat Products

Microchemical studies of the wheat kernel indicate that there are no starch granules in the pericarp, or in the germ or embryo of the mature, resting seed. Despite its prominence and ready identification in the endosperm of wheat, acceptable quantitative data respecting the starch content of wheat and wheat products are none too common. As a matter of fact, the quantitative determination of starch is not altogether simple and easy, and none of the methods thus far proposed may be regarded as altogether precise. Among these methods the following have been most prominent:

(1) Mechanical removal of the starch granules by washing with water, recovering the granules by centrifuging, and weighing.

(2) Acid hydrolysis of the starch, and determining the reducing sub-

⁸³ *Ann.*, **452**, 141 (1927).

⁸⁴ *Ann.*, **458**, 93 (1927).

⁸⁵ *Ann. Rev. Biochem.*, **7**, 51 (1938).

⁸⁶ *Shirley Inst. Mem.*, **14**, 63 (1935).

⁸⁷ *J. Chem. Soc.*, 2277 (1932).

stances as glucose. The determination is usually made upon a sample previously washed with dilute ethanol to remove sugars.

(3) Enzymic hydrolysis of the starch in a sample accorded pretreatment as in (2), followed by acid hydrolysis of the maltose and determining the reducing substances as glucose.

(4) Dissolving the starch in a sample from which the sugars had been removed, using CaCl_2 solution as the starch solvent, and then estimating the starch from the optical activity of the solution as observed with a saccharimeter.

(5) Dispersion of starch by HCl solution, reprecipitation by alcohol, recovery by centrifuging, and weighing (Rask's method).

(6) Colorimetric or photometric (monochromatic light, 590-650 μ) observations of starch-iodine systems, as detailed by Simerl and Browning.⁸⁸

While this monograph is not designed to present complete analytical methods for all the materials under discussion, a comparison of the results of starch analyses made by several methods serves to emphasize the necessity for recording such results in terms of the methods used. Herd and Kent-Jones⁸⁹ reported the results of analyses of several samples of flour, as determined by: I. Ling's malt diastase method, II. Ling, Nanji and Harper's barley diastase method, and III. Rack's acid dispersion method mentioned under (4) above. The results are recorded in Table 51.

Table 51. Results of Analyses of Several Flour Samples for their Starch Content, as Reported by Herd and Kent-Jones.⁸⁹

Flour Sample	Starch as determined by—		
	I Malt diastase method (%)	II Barley diastase method (%)	III Acid dispersion and reprecipitation (%)
Straight run	64.3	65.2	63.2
Flour from "A" roll	67.9	67.3	63.5
Flour from "H" roll	66.9	63.2	57.3
Flour from "J" roll	61.9	62.1	52.3
Flour from 4th break roll	61.3	59.4	56.1
Commercial flours A 407	73.6	73.2	70.6
W/W 88	76.2	74.0	70.1
E 765	68.7	68.1	65.8
L 49	68.3	67.1	65.1
Straight-run baking flour	67.1	65.3	65.0
Straight-run pastry flour	70.2	68.3	67.2

Similar analyses were made of four whole wheat samples, and four English mill offals with the results recorded in Table 52.

Despite the fact that Rask's acid dispersion and reprecipitation method III gave lower results than the malt diastase method in all but

⁸⁸ *Ind. Eng. Chem., Anal. Ed.*, **11**, 125 (1939).

⁸⁹ *J. Soc. Chem. Ind.*, **50**, 15 (1931).

Table 52. Results of Analyses of Wheat and Mill Offal Samples for their Starch Content, as Reported by Herd and Kent-Jones.⁹⁰

Description of sample	Starch as determined by—		
	I Malt diastase method (%)	II Barley diastase method (%)	III Acid dispersion and reprecipitation (%)
Wheats: Manitoba III	52.1	50.2	51.5
English	56.8	54.0	54.2
Plate	49.0	46.0	51.9
<i>Durum</i>	53.6	48.5	54.0
Mill offals: Sharps A	20.2	19.7	16.6
“ B	23.3	23.6	16.9
Bran A	11.1	13.5	5.8
“ B	12.0	14.2	6.5

two of the 19 cases, the Rask method applied to two commercial starch samples gave substantially higher results than the malt diastase method by the order of about 6 per cent. Also it resulted in returning more nearly 100 per cent of the original sample when included in a “complete analysis.” This suggests that the enzymic techniques may have resulted in including substances other than starch.

Using dry grinding in a pebble mill to avoid hydrolysis of starch such as might be effected by heat treatment, Field⁹⁰ observed the specific rotatory power of wheat starch to be 194.7°. The cold-water-soluble portion of wheat starch prepared in this manner was free from phosphorus.

Scheele and Svensson⁹¹ described several methods for the determination of starch in cereals analogous to those just mentioned and applied some of them to wheat and other materials. Polarimetric methods gave the same results as the estimate by difference when a “complete” analysis of wheat starch was made. Results by acid and enzymic determinations were in good agreement and 54 to 58+ per cent of starch was reported in three wheat samples analyzed by them.

An undescribed sample of wheat flour that was analyzed by Hartmann and Hillig⁹² was found to contain 74.4 per cent of starch. The method employed involved boiling, the removal of the native sugar and fat, boiling the residue with about 0.05*N* HCl, and then digesting with pepsin. The paste was then neutralized and further digested with malt extract at about 65°, using three successive portions of fresh extract. The digestate was later treated with HCl at the temperature of boiling water, presumably to hydrolyze the maltose present. Finally the preparation was cooled, neutralized, and the glucose present was determined by the Munson-Walker method.

Hopkins⁹³ developed a convenient method involving the extraction of

⁹⁰ *Proc. Soc. Expt. Biol. Med.*, 25, 711 (1928).

⁹¹ *Z. ges. Getreidew.*, 15, 229; 268 (1929).

⁹² *J. Assoc. Official Agr. Chem.*, 14, 112 (1931).

⁹³ *Can. J. Research*, 11, 751 (1934).

starch with 33 per cent CaCl_2 solution, followed by the observation of its optical activity. This method was applied to wheat starch, and to samples of several wheats and flours with the results recorded in Table 53.

Table 53. Starch Content of Samples Analyzed by Hopkins⁹³ Based upon Optical Gravity of CaCl_2 Solution Extracts.

Description of Sample	Starch (%)
Wheat starch preparation	87.83
Hard spring wheat, No. 1 northern (Marquis)	51.88
Soft winter wheat, Dawson's Golden Chaff	59.05
Flour from hard wheat (Marquis)	68.06
Flour from soft wheat	71.09

Hopkins and Graham⁹⁴ applied Hopkin's⁹³ polarimetric method to the analysis of a series of wheat samples with the results shown in Table 54. There was a tendency for the starch content to vary inversely with the protein content, as one would predict from the physiological considerations involved in the deposition of starch in the developing wheat kernel. Also the sum of starch plus protein decreased with decreasing numerical grade of the wheat.

Table 54. Starch Content of Wheat Samples, as Reported by Hopkins and Graham⁹⁴ Using Hopkins' Polarimetric Method.

Grade of wheat	Starch (13.5% moisture basis)
No. 1 northern	51.88
No. 2 "	52.00
No. 3 "	50.13
No. 4 "	51.03
No. 5 wheat	51.99
No. 6 "	51.45
Feed wheat	51.72
Ontario Marquis	52.08

Hopkins⁹⁵ reported further on the starch content of Canadian wheats. In the instance of average samples of each of the Canadian wheat grades ranging downward from 1 Hard to 6 Special, where the grading factor appeared to be the weight per bushel, the starch content tended to diminish with decreasing bushel weight, ranging from 52.23 per cent in the highest to 47.80 per cent in the lowest grade. In consequence there was a high positive correlation between starch content and specific gravity ($r = +0.86$; 1% point = 0.71), since the last-named factor was conditioned largely by the weight per bushel, and, doubtless, by the size of the individual kernels. Unfortunately, the author did not make that point clear, and any inference that starch content and specific gravity are positively correlated, other characteristics being the same, is unfounded. If

⁹⁴ *Can. J. Research*, 12, 820 (1935).

⁹⁵ *Can. J. Research*, 17, 253 (1939).

these two variables had been contrasted, with weight per bushel and kernel weight held constant, it is probable that a *negative* correlation would have appeared. The same comment would apply to the positive relationship observed by Hopkins between starch content and flour yield in the instance of this set of data.

Cranfield⁹⁶ elaborated upon Jago's⁹⁷ method for the determination of starch in mill offals, using a simple and direct method of removing the starch granules by washing the wetted material while it was enveloped in a bag of flour silk. The starch suspension was made to a standard volume, an aliquot removed, the starch recovered by centrifuging, and weighed after suitable washing. Some typical results of the application of this method are recorded in Table 55.

Table 55. Percentage of Crude Starch in Certain English Mill Streams, as Determined by Cranfield⁹⁶

Grade	Description of streams (English milling systems)	Crude starch (%)
I & II	Fine middlings, middlings, fine sharps	44.7
III & IV	Middlings, fine sharps, sharps, coarse middlings	34.7
V & VI	Sharps, coarse sharps, pollards	26.8
VII, VIII & IX	Bran, ordinary bran	19.6
X	Broad bran	15.8

Starch was determined in several classes of Canadian wheat by Eva, Levi, and Anderson^{97a} using a method which involved extracting the starch with calcium chloride solution, precipitating the dispersed protein with stannic chloride, and observing the solution with a saccharimeter. The specific rotatory power of starch was assumed to be $+200^\circ$. In an extensive collection of hard spring wheat samples from six Canadian inspection offices, and grown during the four crop seasons of 1939 to 1942 inclusive, the mean starch content was 53 per cent. The variability in this collection was not large, considering the range in time, grade, and area represented. Thus 90 per cent of the samples contained between 51.0 and 54.9 per cent of starch. In a collection of *durum* wheats the mean starch content of all grades was 54.1 per cent, and in a collection of Alberta winter wheats the average was 54.4 per cent. In general, starch content and protein content were inversely related, although the data in support of this observation were not presented.

The starch content of several types of American wheats, in per cent, dry basis, was reported by Stark, Kolachov and Willkie⁹⁸ as follows: Soft white winter, 72.1, 72.5; Soft club, 72.3; Soft red winter, 68.4, 70.9; Hard red winter, 62.3, 66.0, 69.1; Dark hard winter, 58.0; Red durum,

⁹⁶ *J. Agr. Sci.*, 6, 102 (1914).

⁹⁷ "The Technology of Breadmaking," p. 814, London, 1911.

^{97a} *Can J. Res.*, 21, 173 (1943).

⁹⁸ *Ind. Eng. Chem.*, 35, 133 (1943).

62.5; Dark northern spring, 59.2, 63.4; Northern spring, 60.9. The determination was made by "diastase-hydrochloric acid modification," according to the authors.

The mineral constituents of wheat starch were determined by Samec⁹⁹ with the following results:

	100 g dry starch contained: g	Equivalents $\times 10^5$
Ash	0.294	
SiO ₂	0.019	63
P ₂ O ₅	0.149	620
SO ₃	0.066	160
CaO	0.042	149
MgO	0.026	129
K ₂ O	0.027	57
Na ₂ O	0.032	100
Fe ₂ O ₃	trace	
Sum of acid equivalents		843
Sum of base equivalents		435

Hygroscopicity of Wheat Starch

Rakowski¹⁰⁰ determined the hygroscopic moisture content of wheat starch in equilibrium with atmospheres of varying vapor pressure. The results are expressed graphically in Fig. 15. It appears that when the

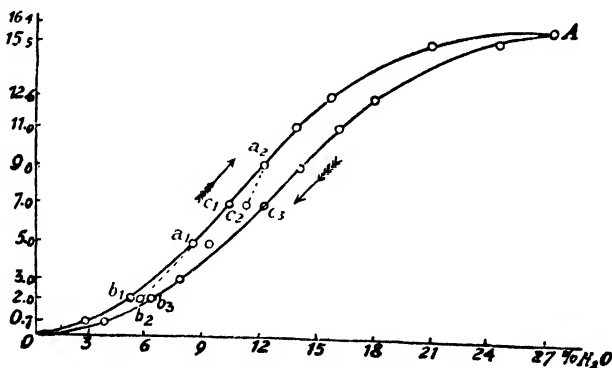


FIG. 15. Vapor pressure in mm. (vertical axis) of starch at different levels of moisture content (horizontal axis) descending from 27 per cent moisture content (lower curve) and ascending to 27 per cent moisture content (upper curve) as reported by Rakowski.¹⁰⁰

hygroscopic moisture content was elevated to about 27 per cent, and then lowered by exposing to progressively drier atmospheres, the moisture content was rather uniformly higher at each level of vapor pressure than when the vapor pressure was ascending. Rakowski¹⁰¹ evolved the for-

⁹⁹ *Biochem. Z.*, **186**, 337 (1927).

¹⁰⁰ *Kolloid-Z.*, **9**, 225 (1911).

¹⁰¹ *Kolloid-Z.*, **10**, 22 (1912).

mula for computing hygroscopic moisture from the observed vapor pressure, which becomes $y - a = a \cdot \sin (\sigma - B)$, in which y is water-vapor pressure in mm, a is a constant which averaged 8.3; $\sigma = \frac{180}{Xm} X$, where

Xm is 27, and X is the estimated moisture content of the starch in per cent. The observed moisture content and the estimate computed from this formula did not differ by more than 0.5 per cent, and was less than that amount in most cases, particularly in the lower levels of moisture content and vapor pressure.

Chapter 9

Sugars

Sucrose was identified in wheat as well as other cereals by O'Sullivan,¹ together with other non-reducing and reducing fermentable sugars which were not identified. Banister, in discussing O'Sullivan's contribution, confirmed that sucrose was recognized in consequence of the ease with which it was inverted, and its non-reducing property. In winter wheat he found 2.57 per cent, in spring wheat 2.24 per cent of the sugar.

The carbohydrates of wheat and flour were studied by Stone.² In a review of the literature he emphasized the prominence of pentosans in the pericarp of wheat; thus 22 per cent of this carbohydrate had been found in bran. After detailing the methods which he employed, Stone reported the carbohydrates in winter and spring wheat samples, and in winter and spring wheat commercial flours that are recorded in Table 56. It is doubtful whether the methods here employed may now be regarded as adequate for this purpose; certainly the starch analyses are decidedly on the low side, and the pentosan method was probably inadequate. Also the percentages of sucrose found in the flour are lower than those reported elsewhere. Stone's findings are of interest from the historical standpoint, however, and served at the time to demonstrate the diversity of types of carbohydrates to be expected in such material.

Table 56. Carbohydrates in Wheat and Flour, as Determined by Stone² on a Dry Matter Basis.

Carbohydrate (%)	Winter wheat	Spring wheat	Winter wheat flour	Spring wheat flour
Sucrose	0.51	0.72	0.20	0.18
Dextrin	0.27	0.41	1.06	0.90
Starch	30.94	30.36	34.04	46.19
Pentosans	4.54	4.37	none	none
Fiber	2.68	2.51	0.25	0.25

The data of Krug, as reported by Wiley *et al.*,³ included determinations of various sugars in wheat and flour. Certain typical values are as follows:

	Reducing sugar (%)	Sucrose (%)	Dextrin (%)
Wheat	0.027	0.330	0.160
Wheat flour	0.014	0.101	0.190
Common market flours	0.021	0.288	0.210
Patent family flours	0.002	0.085	0.200

¹ *Chem. News*, 52, 293 (1885).

² *U. S. Dept. Agr., Office Exp. Stas. Bull.* 34 (1896).

³ *U. S. Dept. Agr., Div. Chem. Bull.*, 13, Pt. 9, 1206 (1898).

The percentages of sucrose here indicated are lower than those reported in more recent times.

Sucrose and raffinose were identified in wheat germ by Frankfurt.⁴ He reported 24.34 per cent of total soluble carbohydrates, and this included 6.89 per cent of raffinose. Presumably the difference was deemed to be chiefly sucrose.

Small amounts of glucose in the range of 0.14 to 0.40 per cent were found in four samples of flour analyzed by Liebig.⁵ When these were digested with water for several hours, additional reducing sugar appeared, which was found to be maltose. In the original flours the sucrose content was between 0.96 and 1.64 per cent. The latter was estimated from the reducing sugars present in an alcoholic extract of the flour following inversion of the sugars with dilute HCl.

Sucrose was isolated from the wheat grain by Schulze⁶ in crystalline form, identifiable by its specific rotatory power, reaction with resorcinol, and hydrolysis by invertase. He failed to recover raffinose as crystals, however. Analysis of wheat germ disclosed 24.34 per cent of water-soluble carbohydrates, chiefly sucrose and raffinose, including 6.89 per cent of the latter.

Sugars in the alcoholic extract (10 per cent ethanol) of flour milled from Canadian wheat were determined by Shutt.⁷ In the flours milled from high-grade wheat the total sugars ranged from 1.04 to 1.14 per cent, while in the flours from lower-grade wheats the total sugar reached 1.48 per cent. Calculated as maltose, the reducing sugars in the high-grade wheat flours ranged from 0.13 to 0.20 per cent. After inversion, calculated as sucrose, Shutt found 0.87 to 0.94 per cent in the same flours.

Sucrose was found by Teller⁸ to be present in ripe wheat to the extent of 1.46 per cent and to constitute 0.35 per cent of patent flour, 4.60 per cent of bran, and 14.60 per cent of the germ of wheat.

The sucrose content of the non-germ portion of the wheat kernel was found by Kretovitch⁹ to be slightly lower than the germ end. Thus in the part representing 22.24 per cent of the kernels, and including the germ, the sucrose content was 2.18 per cent and the reducing sugars 0.21 per cent. The portion without the germ, representing the remaining 77.76 per cent of the grain, contained 1.45 per cent of sucrose, and no detectable reducing sugars. The weighted average of the two fractions thus became: sucrose 1.62 per cent, reducing sugars 0.04 per cent.

Another mechanical fractionation of the kernels was then made by

⁴ *Landw. Vers. Sta.*, 47, 449 (1896).

⁵ *Landw. Jahr.*, 38, 251 (1909).

⁶ *Landw. Vers. Sta.*, 73, 35 (1910).

⁷ *Cent. Exptl. Farm (Ottawa, Canada) Bull.* 80, pp. 19-20 (1908).

⁸ *Proc. 8th Internat. Cong. Appl. Chem.*, VIa, 273 (1912).

⁹ *Biochem. J.*, 27, 1687 (1933).

Kretovitch as follows: (I) the end containing the germ was cut off with a scalpel. This represented 19.06 per cent of the weight of the grain; the rest was then divided into (II) the outer layers of endosperm with the bran coat and aleurone layer, and representing about 24 per cent of the entire kernel, and (III) the inner layers of the endosperm representing about 57 per cent of the layer. The reducing sugar and sucrose content of these three portions of the grain were:

Zone or portion	I	II	III
Reducing sugars	0.0	0.0	0.0
Sucrose	2.96	2.58	0.88

The sharp falling off in sucrose content in progressing from the outer (II) to the inner (III) portions is notable.

Having observed the presence of a sugar in wheat germ which did not yield levorotatory products on hydrolysis, Richardson and Crampton¹⁰ proceeded to recover 15-18 per cent of sugars from de-fatted wheat germ by extracting with hot 80 per cent ethanol, which was about 80-90 per cent sucrose. The remainder was a non-fermentable sugar, non-reducing before hydrolysis, strongly dextrorotatory, and hydrolyzed by yeast invertase to reducing substances. These characteristics resembled those of raffinose.

Raffinose was recovered as its compound with strontium hydrate by Schulze and Frankfurt¹¹ when they worked up their extract of wheat germ. The product was not entirely pure, however, some sucrose being present. On further purification by recrystallization, a preparation was obtained with $(\alpha)_D = +105.5^\circ$, which is in the same range as previously reported for raffinose [$(\alpha)_D = +104.5$]. Also, on oxidation, the preparation yielded 22.2 per cent of mucic acid, as compared with Tollens' recovery of 22-23 per cent.

Synanthrose, presumably identical with the carbohydrate previously recovered under that name from the girasole, was found in rye flour and bran by Muntz,¹² but not in wheat.

"Amylan" was obtained from wheat by O'Sullivan¹³ by first extracting with alcohol (sp. gr. 0.9), and then treating the residue with water at 35-38° for several hours. This aqueous extract was evaporated, and alcohol added to produce a precipitate. This was dried and washed with alcohol, water (I) and cold dilute HCl successively. The washed residue (II) was then dissolved in boiling water, filtered, and precipitated with alcohol acidified with 3-4 per cent HCl. Re-solution and re-precipitation

¹⁰ *Ber.*, 19, 1180 (1886).

¹¹ *Ber.*, 27, 64 (1894).

¹² *Compt. rend.*, 87, 679 (1878).

¹³ *Chem. News.*, 44, 258 (1881).

were carried out at least once, followed by drying. The preparation had the empirical formula $C_6H_{10}O_5$, was levorotatory $(\alpha)_D = -24^\circ$, and was almost soluble in cold, but gelatinized by hot water. On hydrolysis with 5 per cent H_2SO_4 it was converted almost quantitatively into glucose. It was called α -amylan.

The water-soluble fraction recovered at (I) above was purified, dried, and found to have a specific rotatory power of $(\alpha) = -73$, and yielded a birotatory modification of $(\alpha) = -144$. It was called β -amylan. Like the α -form it was hydrolyzed, apparently to glucose.

Wheat (and rye) contained 2 — 2.5 per cent of β -amylan, and not more than 0.1 per cent of α -amylan.

Levosine was the name first applied by Tanret¹⁴ to a carbohydrate isolated from cereals which had the following properties: white, amorphous, dry powder of insipid taste; dissolves readily in water, and in dilute alcohol; slightly, if at all, soluble in 95 per cent alcohol; in aqueous solution it was levorotatory, $(\alpha)_D = -36^\circ$; action of dilute acids increased the rotation to $(\alpha)_D = -76^\circ$; did not reduce Fehling's solution; diastase was without action; levulose crystals [$(\alpha)_D = -89^\circ$] were recovered from the hydrolyzate; on treatment with fuming nitric acid a white mass was recovered, non-crystallizable, insoluble in water and with a specific rotatory power in alcoholic solution $(\alpha)_D = +15.6^\circ$.

For levosine, Tanret¹⁵ proposed the formula $C_{48}H_{40}O_{40}$, or $(C_{12}H_{10}O_{10})_4$. In addition to the other properties described above, mention was made of the fact that, upon treatment with nitric acid, it was converted into oxalic acid without any intermediate formation of mucic acid.

The prominence of levosine in wheat kernels early in their development was emphasized by Colin and Belval,¹⁶ who found 6.07 per cent on June 12, 1922, followed by a steady decrease to 0.85 per cent on July 25. A collection of wheats of several genetic species was analyzed by Colin and Belval¹⁷ with the following results:

	Reducing sugars (%)	Sucrose (%)	Levosine (%)
Common Engrain	0.19	0.30	0.31
Allied wheat	0.10	0.50	0.25
Epeutre	0.14	0.19	0.50
Amidonnier	Trace	0.60	0.69
Hard wheat of the Wallaga	0.27	0.39	0.74
<i>Triticum polonicum</i>	0.30	0.60	1.35
<i>Triticum dicoccoides</i>	0.33	0.16	1.26

¹⁴ *Bull. soc. chim. (S)*, 5, 724 (1891).

¹⁵ *Compt. rend.*, 112, 293 (1891).

¹⁶ *Compt. rend.*, 177, 343 (1923).

¹⁷ *Ibid.*, 177, 973 (1923).

It appeared that the non-*vulgare* wheat species contained more levosine than the common wheats.

Of the total glucides representing about 1 per cent of wheat flour, Colin and Belval¹⁸ found 0.10 to 0.15 per cent of reducing sugars, about twice this amount of sucrose, and the remainder was deemed to be levosine, the fructoside discovered by Tanret. Bran and germ were observed to be free from levosine, the former being rich in sucrose, the latter in sucrose and raffinose. They contend that the earlier analyses which recorded the non-reducing glucides as sucrose were in error. It was recognized that maltose was formed by diastatic action in the dough when water was added to flour. The specific rotatory power of a protein-free alcoholic extract of dough was only $+80^\circ$, instead of $+138^\circ$, which is the recognized value for anhydro-maltose, and this was interpreted to indicate the persistence in the dough, and the presence in such an extract of levorotatory levosine. Moreover, on hydrolysis of the glucides in such an extract, the dextrorotatory power falls, indicating the appearance of fructose as a product of hydrolysis. As usual with such fructosides, the levosine of flour was not completely fermented by yeast in the normal short-fermentation of a bread dough.

In a typical flour, Colin and Belval found 0.10 per cent of hexoses, 0.25 per cent of sucrose and 0.60 per cent of levosine. After such a flour was converted into a dough and fermented with yeast, no hexose or sucrose remained, but the dough contained 0.34 g of levosine and 0.92 g of maltose per 100 g of flour in the dough.

In a later publication Colin and Belval¹⁹ recite the description given to this levosine by Tanret²⁰ as follows: white, amorphous, insipid, freely soluble in water, nearly insoluble in strong alcohol, levorotatory (α)_D = -36° , non-reducing, non-fermentable, easily hydrolyzed by dilute acids, yielding chiefly fructose and a small quantity of glucose, the mixture having a rotatory power of -76° .

Early in the development of the wheat kernel the levosine is three times as abundant as starch, but becomes less prominent as the grain ripens. Rye and barley, as well as wheat, contain levosine, but it disappears from oats as they approach maturity, and is not found in maize, sorghum or rice kernels.

In ripe wheat, barley and rye kernels the percentages of levosine reported by Colin and Belval were 0.3, 0.5, and 1.6 respectively. Since there was none in the bran and shorts, the percentage in flour was greater than that in the whole kernel, reaching 0.6 per cent in wheat flour, and 3.1

¹⁸ *Compt. rend.*, 200, 2032 (1935).

¹⁹ *Bull. soc. chim.* (6), 2, 1907 (1935).

²⁰ *Ibid.*, (3), 5, 724 (1891).

per cent in rye flour. Incidentally, wheat germ was here reported as containing 4 per cent of raffinose. A graph was presented showing the concentration of various sugars in leavened dough during a four-hour fermentation period, and the levosine was again represented as diminishing to about half of the original content.

The extraction and recovery of levosine from flour was described by Colin and Belval,²¹ involving treatment with 75 per cent alcohol, purification with a little baryta, filtering, concentration, and precipitation of the fructosan with an excess of baryta. Reprecipitation from its solution yielded a white powder, $[\alpha]_D = -36^\circ$. On hydrolysis it yielded chiefly fructose, 9 parts, to 1 part of a hexose that apparently was glucose. The levosine was described as a holo-gluco-fructoside. The enzymes of the powdered mycelia of *Aspergillus niger* effected its hydrolysis, but the distillery *mucors* scarcely effected it. Baker's yeasts attacked it more vigorously than brewer's yeasts. Flour enzymes did not hydrolyze it in a period of several hours.

Carbohydrates soluble in 60 per cent alcohol were extracted from nine sets of mill products by Cugnac,^{22, 23} freed from pentosans and other interfering non-sugar constituents by defecation, and subjected to analysis. Three major components were recognized, (1) reducing sugars, (2) sucrose, and (3) non-sucrose polysaccharide(s) which was not hydrolyzed by sucrose, and was assumed to be the levosine of Tanret. The mill products examined by Cugnac included (a) a white flour representing 65 per cent of the wheat, (b) a dark flour representing the next 15 per cent of extraction, and (c) feed or bran representing the residual 20 per cent of the wheat. In every case the dark flour contained the largest percentage of total sugar. The sucrose content of the white flour was lower than in the other mill products, ranging from 0.06 to 0.53 per cent, and averaging 0.21 per cent. The dark flour and the bran were not very different in sucrose content, averaging 1.08 and 1.05 per cent respectively. Insofar as the non-sucrose (presumably levosine) content was concerned, the white flour contained the least. The range was from 0.39 to 1.51 per cent, with an average of 0.71 per cent. In the dark flour and the bran, averages of 2.66 and 2.02 per cent were encountered.

The reducing sugars of flour, amounting to only about 0.12 per cent, were estimated by Geoffroy²⁴ to be comprised of glucose and fructose in the ratio of 3:1. About 0.25 per cent of sucrose was present in the flour which he analyzed, as well as a levuloside which was not completely fer-

²¹ *Bull. soc. chim. biol.*, 17, 1040 (1935).

²² *Ann. agron.*, 2, 375 (1932).

²³ *Bull. soc. bot. France*, 79, 231 (1932).

²⁴ *Bull. soc. chim.*, 51, 1491 (1932).

mentable under the conditions of panary fermentation. Geoffroy²⁵ also observed that the sucrose increased substantially and levosin increased slightly from the center to the periphery of the endosperm of the wheat kernel. Wheat gums were found, about 0.2 per cent being present in flour. These gums appeared to consist largely of araban and glucosan. A later study by Geoffroy²⁶ confirmed his earlier conclusion that flour contains a levosin, in the amount of 0.2 per cent, which appeared to be a tetraholoside. While its aqueous solution was not fermented by a baker's yeast, it did tend to disappear during the fermentation of a bread dough. Examination of another series of flours by Geoffroy²⁷ disclosed less than 0.5 per cent of sucrose, less than 1 per cent of levosin, and 0.1-0.4 per cent of glucose.

In the earlier papers by Guillemet and Schell^{28, 29} dealing with the sugars in flour, they give the impression that the principal fermentable non-reducing sugar of flour is sucrose, and the total concentration of sugars thus described is in the range of 0.9 to 1.5 per cent. Later, however, these investigators³⁰ reported an estimated concentration of 0.2 to 0.7 per cent of sucrose in flour, a small amount of hexose, and the remainder of the 1 to 2 per cent of fermentable carbohydrates other than maltose appeared to be a mixture of complex polysaccharides, including fructoholosides. Guillemet³¹ also observed that in the absence of an activator present in the aqueous extract of macerated yeast, previously called *Factor Z* by Euler, levosin was only fermented slowly, but with *Factor Z* present 30 per cent was fermented in 24 hours under the conditions of the experiment.

In view of the apparently conflicting views expressed in the literature concerning the fermentability of levosine by baker's yeast, the present author suggests that the disappearance of levosine during bread dough fermentation may be the consequence of the presence in such dough of dead and disintegrated yeast cells which contribute some *Factor Z* to the dough solution.

Wheat flour was found by Genevois and Pavloff³² to contain 2-5 per cent of fermentable sugars, and 1 per cent of maltose was present in aqueous extracts after 1 to 5 hours.

Sprouted wheat contains more soluble carbohydrates, including sugars, than normal wheat as shown by Harcourt.³³ Thus he allowed wheat to

²⁵ *Bull. soc. chim. biol.*, 16, 1297 (1934).

²⁶ *Ibid.*, 17, 848 (1935).

²⁷ *Ibid.*, 17, 1351 (1935).

²⁸ *Compt. rend. soc. biol.*, 116, 1402 (1934).

²⁹ *Compt. rend.*, 198, 1083 (1934).

³⁰ *Bull. soc. chim. biol.*, 18, 1132 (1936).

³¹ *Compt. rend.*, 201, 1517 (1935).

³² *Compt. rend.*, 200, 690 (1935).

³³ Report Ontario (Canada) Agr. College, p. 81, 1911.

germinate until the sprouts were (II) one-half, (III) one and one-half, and (IV) two and one-half times the length of the kernel, and the chemical composition of such sprouted wheats was compared with (I) normal wheat. Both soft winter wheats grown in Ontario and hard spring wheats grown in Manitoba were used in this study. The analyses, insofar as they involved the carbohydrates, were reported by Harcourt as shown in Table 57. It is the usual assumption that the additional sugar which appears on germination is maltose, produced by enzymic hydrolysis of the starch. This is supported by the evident decrease in starch content, and the increase in total sugars which occurred during the course of this particular experiment. There was some increase also in the percentage of dextrin-like substances as germination progressed, which probably represent products of starch degradation.

Table 57. Carbohydrates in Normal and Sprouted Wheat, as Reported by Harcourt.³³

Sample	Carbohydrates in wheat		
	Total sugars (%)	Soluble starch and dextrin (%)	Starch (%)
Ontario winter wheat			
Normal (I)	0.90	3.46	65.3
Sprouted (II)	1.44	3.22	64.8
Sprouted (III)	1.63	4.03	63.7
Sprouted (IV)	2.83	4.23	62.9
Manitoba spring wheat			
Normal (I)	1.02	3.66	64.4
Sprouted (II)	1.48	3.55	63.7
Sprouted (III)	2.39	4.62	61.9
Sprouted (IV)	3.02	5.22	59.8

Reducing sugars in a series of 33 samples of baker's flours were determined by Blish, Sandstedt and Astleford.³⁴ Using the picric acid colorimetric method, and computing the results as maltose, a range of from 0.04 to 0.19 per cent with an average of 0.14 per cent was reported. When the increase in reducing power of a flour extract after hydrolysis with HCl was assumed to be due to sucrose only, the percentage of sucrose thus determined ranged from 1.00 to 1.74 per cent, with an average of 1.25 per cent. The variability was not as great as might be inferred from the range, however, since the standard deviation of these 33 determinations as computed by the present author was only $\sigma = 0.1667$.

The comparative ease with which sucrose is hydrolyzed by dilute H_2SO_4 was used by Sandstedt³⁵ to distinguish it from maltose and to effect its determination quantitatively in the presence of the latter. Conditions for hydrolysis were described which completely inverted the

³⁴ *Cereal Chem.*, 9, 378 (1932).

³⁵ *Ibid.*, 14, 767 (1937).

sucrose, and without an appreciable hydrolytic effect on any maltose that was present.

Levulose in the amounts of 0.34 to 0.46 per cent was found by Rice ³⁶ in bread to which no sucrose or levulose had been added. This was interpreted to indicate the presence of nearly 1 per cent of sucrose or its equivalent as invert sugar in the original materials. Incidentally, Rice's analyses of bread made with various sugars indicate that yeast manifests a preference for glucose over fructose when both are present in the same proportions. Consequently the glucose was fermented at a greater rate and was present in smaller amounts in the baked loaf; in fact, it nearly disappeared unless provided in large proportions, or a sharp excess over the fructose.

³⁶ *Ibid.*, 15, 672 (1938).

Chapter 10

Gums, Pentosans, Hemicelluloses, and Cellulose

The presence of gums in cereal grains was observed by von Bibra,¹ and Ritthausen;² and later Ritthausen³ concluded that they were polymerized hexoses. Haas and Hill⁴ considered it more probable that they were polymerized mannose, however. Osborne evidently encountered gums in purifying soluble proteins, and Hoffman and Gortner⁵ obtained a gum from their 5 per cent K_2SO_4 extracts of flour which was a polymerized carbohydrate.

Dilute KOH extracts a gummy material from wheat bran which was shown by Gudkow⁶ to yield large amounts of pentose sugar; this was confirmed by Stone and Tollens,⁷ who recovered a phenylosazone of arabinose from the hydrolyzate, as did also Steiger and Schulze.⁸

Pioneer work on the pentosans and their estimation from the furfural resulting from distillation with HCl solution may be credited to Tollens and his collaborators,⁹ and to Schulze and co-workers¹⁰ in the period around 1890. Tollens¹¹ found 24.7 per cent of "pentaglucoses" in wheat bran, as determined from the yield of furfural.

A furfural-containing constituent of wheat bran was identified by Steiger and Schulze,¹² although they recognize that the earlier work of Gudkow¹³ established that such substances were present in bran. Arabinose was identified in the hydrolyzate of the parent substance, with an $(\alpha)_D = +104.2^\circ$, and yielding an osazone characteristic of that pentose. They did not agree that the mother liquor from the arabinose recovery may have contained xylose, since it had a low $(\alpha)_D$ of the order of $+43^\circ$.

The gummy material recovered from wheat, as well as from barley and malt, by Lintner and Düll¹⁴ was hydrolyzed with dilute HCl, and only galactose and xylose could be identified as their osazones. No fermenta-

¹ "Die Getreidearten und das Brot," Wilhelm Schmidt, Nürnberg, 1861.

² *J. prakt. Chem.*, **99**, 439 (1866).

³ *Ibid.*, **102**, 321 (1867).

⁴ "An Introduction to the Chemistry of Plant Products," Vol. I, London, 1928.

⁵ *Cereal Chem.*, **4**, 221 (1927).

⁶ *Z. Chem. (2)*, **6**, 360 (1870); *Ber.*, **3**, 425 (1870).

⁷ *Ann.*, **249**, 227 (1888).

⁸ *Ber.*, **23**, 3110 (1890).

⁹ *Ber.*, **23**, 1751 (1890); **24**, 694 (1891).

¹⁰ *Ibid.*, **23**, 3110 (1890).

¹¹ *J. Landw.*, **40**, 11 (1892).

¹² *Ber.*, **23**, 3110 (1890).

¹³ *Z. Chem. (2)*, **6**, 360 (1870); *Ber.*, **3**, 425 (1870).

¹⁴ *Z. angew. Chem.*, **4**, 538 (1891).

tion with yeast could be effected in the neutralized hydrolyzate. The elementary analysis of the gum gave results which agreed well with the theoretical if it was assumed that the gum consisted solely of a condensate of the two sugars mentioned.

Wheat straw was found by Allen and Tollens¹⁵ to yield a gum which, when extracted with NaOH solution and precipitated with alcohol, was converted into xylose on hydrolysis, and on distillation with HCl gave much furfural. No reaction for methyl furfural, as a product of distillation of methyl pentosans, could be obtained on distillation of wheat bran with HCl by Widtsoe and Tollens.¹⁶

"Hemicelluloses" of wheat bran were studied by Schulze.¹⁷ They were found to yield arabinose on hydrolysis, crystalline prisms being recovered, having a specific rotatory power of $+104.2$. Xylose was also identified in the products of hydrolysis, but neither galactose nor glucose was encountered. Accordingly, Schulze decided that araban and xylan were among the characteristic constituents of wheat bran.

Ritthausen¹⁸ directed Effront's attention to the fact that thirty years before Effront announced the recovery of a carbohydrate, "carubin," from St. John's bread, he¹⁹ had described a substance prepared from rye flour and bran that without doubt was identical with carubin. Tollens had applied the name "secalin" to gum recovered from rye. It was soluble in acetic acid, and was precipitated from its solution by addition of a large volume of strong alcohol. On hydrolysis by acid and heat it yielded 82 per cent of reducing sugar calculated as dextrose, but it was not determined whether this sugar was actually dextrose, galactose, or arabinose.

On extending his studies of the gums of wheat, Geoffrey²⁰ described the araban of flour. This was a polyarabinoxylolide, yielded a viscous solution in water, $[\alpha]_D$ about -50° . It was slowly hydrolyzed by dilute H_2SO_4 at 85° , yielding arabinose. It was not acted upon by yeast. The araban did not dissolve in 70 per cent ethanol.

Pentosans were found in whole wheat by Teller²¹ to the extent of 7.43 per cent, and in flour, bran and germ at the levels of 2.60, 23.73 and 4.90 per cent respectively.

The significance of the uronic acids in the constitution of plant gums was overlooked by the earlier investigators. This led Norris and Preece²² to reinvestigate the gums of wheat bran, with the result that they recov-

¹⁵ *Ber.*, 23, 137 (1890).

¹⁶ *Ber.*, 33, 143 (1900).

¹⁷ *Z. Physiol. Chem.*, 16, 387 (1892).

¹⁸ *Chem. Zeit.*, 21, 717 (1897).

¹⁹ *J. prakt. Chem.*, 102, 321 (1867).

²⁰ *Bull. soc. chim. biol.*, 19, 60 (1937).

²¹ *Proc. 8th Internat. Cong. Appl. Chem.*, VIa, 273 (1912).

²² *Biochem. J.*, 17, 493 (1923).

ered four fractions from their crude product, two of which yielded sugars and hexuronic acids on hydrolysis, while the other two yielded only sugars, chiefly pentosans.

A re-examination of the gum recovered from flour by Hoffman and Gortner⁵ and other gums prepared by Freeman were reported upon by the latter.²³ He recovered a yield of 4 g from 1500 g of a clear grade wheat flour on applying the following procedure: extraction with half-saturated $(\text{NH}_4)_2\text{SO}_4$ solution, full saturation with the same salt to produce a coagulum which was recovered by filtration, dispersion of the coagulum in water, dialysis to remove all sulfates, evaporation, and reprecipitation by pouring into strong ethanol. This precipitate was dispersed in water and boiled to remove heat-coagulable proteins, followed by filtration. The filtrate containing the gums was then dialyzed till sulfates were removed, evaporated to a syrup, and again precipitated by pouring into strong ethanol. The coagulum was recovered by filtration, washed with ethanol, ether, acetone, and the latter removed by drying at 100° .

On analysis the wheat gum thus prepared from *vulgare* and *durum* wheat species was found to contain from 5.0 to 19.7 per cent of protein, and 67.2 to 82.8 per cent of pentosans. No uronic acids, galactose or fermentable sugars were detected. The protein encountered was assumed to be an impurity, rather than a constituent part of the gum substance.

When a sample of wheat gum was hydrolyzed at 80° with 4 per cent H_2SO_4 for several hours, the hydrolyzate contained reducing substances equivalent to about 90 per cent of glucose. This was evaporated after neutralizing, mixed with strong ethanol, the alcoholic solution evaporated under reduced pressure to a small volume, seeded with *l*-arabinose crystals and kept in a refrigerator for 2 weeks. The crop of crystals thus recovered had an $(\alpha)_D = +101.4^\circ$ (for *l*-arabinose $(\alpha)_D = +104^\circ$), gave a negative Bertrand test for xylose, and yielded a phenylosazone with a melting point of 160° , which is the temperature at which both arabinosazone and xylosazone are reported to melt. Freeman evidently felt that the evidence for the absence of xylose was not clear, however, since the hydrolyzate of such a gum had an $(\alpha)_D = +49.3^\circ$, based upon the quantity of pentose sugar estimated to be present, and this suggests the presence of something other than arabinose. As a matter of fact, one specimen of gum prepared from *durum* wheat flour gave evidence of being a pure xylan.

The gum preparations were very strongly hydrated in the presence of water, and it was estimated by applying Kunitz's formula that each gram

²³ *Cereal Chem.*, 9, 506 (1932).

of gum in the sol state occupied a volume of about 9 cc, which means a hydration of the order of at least 800 per cent. This would account for the extremely high viscosity of dilute aqueous solutions of these gums.

Gums were recovered from wheat by Rotsch and Pietz²⁴ upon treating a suspension of fine wheat meal in water with sodium tungstate and sulfuric acid, and precipitating the gums with alcohol or acetone. A stringy precipitate was recovered from wheat by the alcohol treatment, as contrasted with a flaky precipitate from rye. The wheat gum precipitate formed more gradually on treatment with acetone, and was less inclined to be stringy.

The wheat gum contained no nitrogen and reacted negatively with Millon's and other protein reagents, and with Fehling's solution. After hydrolysis with dilute H_2SO_4 the product reduced Fehling's solution; distillation with 12 per cent HCl resulted in a production of furfural, and a positive reaction with the Rosenthal reagent, acetone and HCl suggested the presence of methyl pentosans. Its water solution treated with α -naphthol and concentrated H_2SO_4 gave a red-violet ring. Orcin-hydrochloride, and corallin-soda did not effect any coloration. On the other hand Ruthenium-red gave an intense red coloration.

There was one striking difference between wheat and rye gums, however, and that was in respect to their specific rotatory power. The rye flour gum was distinctly levorotatory, ranging from -0.87° to -1.12° in the instance of filtrates obtained from 20 g of flour. Similar wheat preparations gave no rotation or up to -0.17° . This difference was made the basis of a proposal for the estimation of the percentage of rye flour (x) in a wheat-rye mixture, involving the formula:

$$x = \frac{100(p - w)}{R - w},$$

where p is the observed rotation of the filtrate from the flour mixture, w the rotation of the pure wheat flour extract, and R the rotation of the pure rye flour. Average values for w and R could be substituted when the flours in the mixture were not available.

Integuments of wheat were found by Notkina and Lifschitz²⁵ to consist chiefly of lignin 12 per cent, tissue 20 per cent, and hemicellulose 40 per cent. The hemicellulose was comprised of 3.0 per cent methyl pentosans, 8.0 per cent uronic acids and 80-85 per cent pentosans.

Hemicelluloses of flour were removed by Clayson and Schryver²⁶ by making a paste of gliadin-free flour, digesting with taka-diastrase three

²⁴ *Mühlenlab.*, 9, 105 (1939).

²⁵ *Ukrain. Khim. Zhur.*, 11, *Wiss.-Tech. Teil* 173 (1936).

²⁶ *Biochem. J.*, 17, 493 (1923).

times to remove the starch, extracting the glutenin from the residue with 0.1 per cent NaOH, and finally treating the resulting residue with cold 4 per cent NaOH. Reference is made later to the characteristics of the undissolved material.

The hemicellulose solution in 4 per cent NaOH was then acidified with acetic acid, which resulted in its precipitation. This was washed successively with water, alcohol, ether, and then air-dried. The fine white powder which resulted dissolved readily in boiling water, from which it reprecipitated on cooling. It gave the blue coloration with iodine that is regarded as characteristic of starch. As appeared from the method of its preparation, it resists digestion with taka-diastase. It did not reduce Fehling's solution, but when hydrolyzed with 1 per cent H_2SO_4 it underwent hydrolysis, yielding glucose. Its specific rotatory power was $(\alpha)_D = +150^\circ$.

From a "straight run" flour of 73 per cent extraction, a yield of 5.4 per cent of hemicellulose was recovered, while from a short patent representing 23 per cent extraction the yield was only 3.6 per cent.

Referring back to the residue from the extraction of the hemicellulose from the protein-free flour, after removal of the hemicelluloses in cold 4 per cent NaOH, a yellowish amorphous substance remained undissolved. This contained a small amount of "cyto pectic acid," which could be extracted from it by 0.5 per cent ammonium oxalate solution. The remainder consisted of cellulose which dissolved completely in zinc chloride-HCl solution.

From purified wheat starch Schryver and Thomas²⁷ obtained a yield of 1.65 per cent of hemicellulose, which contained 2.1 per cent of nitrogen. On correcting for the apparent protein content the net yield became 1.35 per cent.

On hydrolysis with dilute acids the hemicellulose was converted almost quantitatively into glucose, which was identified by its osazones, specific rotatory power, and failure to react for pentoses, fructose and other sugars.

Hemicelluloses of wheat bran were extracted by Norris and Preece²⁸ by an elaborate process which involved (1) 0.5 per cent ammonium oxalate solution at 90° for 2-3 hours, followed (2) by 1 per cent NaOH solution in 50 per cent ethanol to remove the pectin and lignin first. The residue was treated with 4 per cent NaOH solution to remove the hemicelluloses. On acidifying with acetic acid, "Hemicellulose A" was precipitated in a quantity equivalent to 2 per cent of the bran. From the filtrate "Hemicellulose B" was precipitated by a half volume of acetone.

²⁷ *Biochem. J.*, 17, 497 (1923).

²⁸ *Biochem. J.*, 24, 59 (1930).

It represented 1.25 per cent of the bran. "B" was then further fractionated by dissolving it with constant stirring in boiling water, cooling, adding concentrated NaOH to the equivalent of 4 per cent, and mixed Fehling's solution. "Hemicellulose B₁" was precipitated as a copper complex from which it was freed by decomposing with HCl and precipitating with acetone.

"Hemicellulose B₂" was recovered by adding acetone to the filtrate from the Fehling's solution just described.

"Hemicellulose C₂" (so termed because of the analogy to "B₂") was present in the filtrate from the original precipitation of "B" with acetone. On the addition of more it precipitated, and was dissolved in 4 per cent NaOH; Fehling's solution was then added, together with a half volume of acetone, and the resulting precipitate, after decomposing with HCl, was reprecipitated and called "C₂". The yield was 1.25 per cent of the original bran.

On hydrolysis these four hemicellulose preparations yielded the following sugars or sugar acids:

Hemicellulose A: xylose, arabinose
Hemicellulose B₁: xylose, uronic acid
Hemicellulose B₂: glucose
Hemicellulose C₂: arabinose, uronic acid

Pectic materials were identified in cereal grains by Nanji and Norman²⁹ but only in low concentrations. In wheat, the ammonium oxalate extraction method indicated the presence of 0.61 per cent, but none of this was present as free pectin; it was in the form of pectate.

Cellulose content of nearly 4000 Rumanian wheat samples was reported by Zaharia,³⁰ and these data were later subjected to statistical analysis by Berczeller and Wastl.³¹ The percentage of cellulose was less variable, relatively, than protein or ash, but more variable than fat content. When the samples of six crops from 1900 to 1905 inclusive were combined in a single collection, the coefficient of variation of the cellulose content was 9.8 per cent, as computed later by Immer;³² the modal class was the one averaging 2.65 per cent of cellulose when the samples were grouped in classes having a range of 0.1 per cent (water-free basis), and the mean was 2.56 per cent.

²⁹ *Biochem. J.*, **22**, 596 (1928).

³⁰ "Le blé Roumain," 581 pp., Bucharest, 1910.

³¹ *Biochem. Z.*, **177**, 168 (1926).

³² Private communication, July, 1941.

Chapter 11

Lipids, Phospholipids and Sterols

Any of the so-called fat solvents extracts a variety of substances from wheat or its mill products. This follows because, among the simpler triglycerides of the fatty acids that are present, there is doubtless a mixture of mixed glycerides. All these are soluble in the conventional fat solvents, such as di-ethyl ether or petroleum ether. The situation is further complicated by the fact that substances other than simple tri-glycerides of fatty acids are either soluble in the fat solvents, or are dissolved in the mixture of fat and solvent that results when the latter is added to the comminuted material. These solvents are not invariably used in a pure state either, but have been employed in mixtures as, for example, di-ethyl ether and ethanol. Again, they have been applied successively, as in the instance of pre-treatments with alcohol, followed by ether. Also, as will be mentioned later, fairly complicated manipulations have been introduced, such as pre-treatment with acids, alkalis and enzymes, as well as multiple treatments with a single solvent, or hot refluxing before the final extraction. It is not surprising that uniform results have not appeared as far as either the quantity extracted or the chemical composition of the extract is concerned. Consequently each preparation must be considered in the light of its previous history, that is, of the various manipulations, and the solvent or solvents employed.

For convenience in the presentation of this material, it will be divided into four major sections: (I) General, including the relative quantities of fats in wheat and flour, (II) Properties and chemical constitution of the glycerides, (III) Phospholipids, and (IV) Sterols. The separation of the material under these sections is not exact, to be sure, since some overlapping is inevitable in the instance of certain studies.

SECTION I: GENERAL

Wheat-germ oil recovered by de Negri¹ was soluble in ether, benzene, chloroform, and carbon tetrachloride; insoluble in cold anhydrous alcohol, but soluble in 30 parts of hot alcohol. At 65° it dissolved in an equal amount of acetic acid. The fat became rancid very easily, and on storage the equivalent of acidity increased from 5.65 per cent in the fresh oil to

¹ *Chem. Ztg.*, **22**, 976 (1898).

43.86 per cent at the end of a year. Some of the constants of this oil are recorded in Table 60.

In addition to the solubilities of wheat-germ oil observed by de Negri, it was noted by Frankforter and Harding² that it was soluble in carbon disulfide and in acetic anhydride, and fairly soluble in methyl alcohol.

About two-thirds of the fat (ether-extract) of the wheat kernel was found by Jacobs and Rask³ to be in the germ half of the kernel. They estimated that, of the total fat in the kernel, about half was in the bran, including the aleurone layer, nearly two-fifths in the starchy endosperm, and about 11 per cent in the germ.

A preference for the fat content over the ash content as an index for flour grade was indicated by Cerkez,⁴ in the instance of flours ground between mill stones. For the several flour grades he proposed the following basis of classification:

Numerical designation of flour grade	Limits of fat (%)
0	0.60-0.75
1	0.76-1.05
2	1.06-1.15
3	1.16-1.25
4	1.26-1.45
5	1.46-1.62
6	1.63-1.84
7	1.85-2.50
8	2.51-3.45

With few exceptions the fat content was as satisfactory an index of refinement as the ash content in the instance of the flour streams of the Minnesota State Testing Mill examined by Bailey.⁵ The fat and ash content of some representative flour streams are shown in Table 58.

Table 58. Percentage of Ether Extract or Crude Fat and of Ash in Certain Flour Mill Streams, as Reported by Bailey.⁵

Flour stream	Ash (%)	Ether extract (%)
First break	0.69	1.69
Third break	0.51	1.64
Fifth break	1.00	2.06
First middlings	0.40	1.47
Fifth middlings	0.51	1.70
Low grade	1.81	3.32

In a collection of twenty samples of American wheats representing a wide variety of types and market classes analyzed by Sullivan and Near,⁶ the lipid content ranged from 2.30 to 3.84%. The two highest values

² *J. Am. Chem. Soc.*, **21**, 758 (1899).

³ *J. Ind. Eng. Chem.*, **12**, 899 (1920).

⁴ *Z. angew. Chem.*, **1895**, 668.

⁵ *Minn. State Dept. Agr. Bull.* **23** (1923).

⁶ *J. Am. Chem. Soc.*, **49**, 467 (1927).

were registered in the instance of amber *durum* and red *durum* wheats. The ratios of lipid : gluten, and of lipid : protein were computed, and these authors contended that valuable information was thus gained concerning the character of the gluten and the (baking) quality of the wheat. High ratios were encountered in the wheats of inferior quality, in a general way.

In a collection of 40 flour samples averaging 0.456 per cent of ash (dry basis), Denigès, Dubaquié, Labat and Danguoumau ⁷ found an average of 0.95 per cent of fat. The latter was not highly variable.

Flours milled from soft white, soft yellow, and hard wheats were extracted with ether by Arany,⁸ and the contents of dry matter in these extracts were 2.06, 1.43, and 1.85 per cent respectively. In ether extracts of flours treated with 85 per cent ethanol, there were 1.73, 1.12 and 0.72 per cent.

Only part of the "fat" could be extracted from wheat by Cormack,⁹ using the Soxhlet apparatus with ether as the solvent (I). If the extracted residue was treated with 0.2 per cent HCl and 0.05 per cent pepsin for 24 hours, dried, and re-extracted with ether the yield of crude fat was increased. Also when the meal was first digested with HCl and pepsin (II), then dried and extracted, the yield of fat was increased over dried extraction. The differences were even greater when applied to bread rather than to wheat meal. Characteristic effects of the enzymic digestions are shown in the following tabulation:

	Fat in bread and meal (%)	
	Soxhlet extraction	Extraction after preliminary digestion
Wheat meal	1.65	1.99
English white bread	0.27	0.61
Scotch brown bread	1.40	2.80

The percentages of fat extracted from flour, germ, and bran by different solvents and extraction methods were disclosed by the work of Herd and Amos.¹⁰ In general, ethyl ether extracted slightly more crude fat than did petroleum ether. Previous hydrolysis with alkali, or acid, or pretreatment with 70 per cent ethanol (designated here as alcohol hydrolysis, last column) tended to increase the percentage of fat extracted, as shown by the data in Table 59.

When alcohol-ether extraction was employed to recover lipoids from wheat, the average was 1.85 per cent, as compared with 1.20 per cent extracted by ether alone in the instance of four varieties analyzed by

⁷ *Bull. soc. chim.* (5), 3, 1173 (1936).

⁸ *Mezőgazdasági Kutatások*, 13, 9 (1940). *Chem. Abs.*, 34, 3380 (1940). Original not seen.

⁹ *Biochem. J.*, 20, 1052 (1926).

¹⁰ *Cereal Chem.*, 7, 251 (1930).

Table 59. Fat Content of Flour, Bran and Germ, as Determined by Various Methods and Reported by Herd and Amos;¹⁰ Dry Matter Basis.

Material	Petroleum ether (%)	Ethyl ether (%)	Alkaline (%)	Acid (%)	Alcohol ("lipoid") (%)
Straight flour	—	1.53	1.90	1.98	—
Patent flour	1.16	1.20	1.64	1.70	1.99
Bran	4.18	4.36	4.34	6.73	4.99
Germ	7.82	8.26	8.80	9.62	10.31

Pelshenke and Schmidt.¹¹ In another series of samples the average lipid content of twelve German wheat samples was 1.46 per cent, as contrasted with only 0.77 in the flour milled from the same samples.

Fat content of the nearly 4000 samples of Rumanian wheat analyzed by Zaharia¹² was found by Berczeller and Wastl¹³ to be less variable than protein, cellulose, or ash content. For the data of the six crop seasons 1900 to 1905 combined, the coefficient of variation was 7.6 per cent, as computed later by Immer.¹⁴ The modal value for the 6 seasons combined was the class averaging 1.85 per cent of fat (water-free basis) when the data were arranged in groups with a 0.1 per cent range in each group or category, and the mean was also 1.85 per cent.

SECTION II: PROPERTIES AND CHEMICAL CONSTITUTION OF THE GLYCERIDES

Three samples of wheat germ were analyzed by Frankforter and Harding¹⁵; these contained 9.35, 15.6 and 13.75 per cent of oil, respectively. After 35 days of drying at ordinary temperatures the oil gained only 0.37 per cent in weight, while linseed oil gained 7.94 per cent. In another experiment the wheat-germ oil actually lost weight slightly. Its index of refraction was 1.47878 at 20°, decreasing gradually as the temperature was elevated to 1.4586 at 80°. When the "paracholesterol" was removed from the crude ether extract by treating with alcohol, the refractive index at 20° was 1.48325. Two absorption bands were observed, (I) from a point midway between Fraunhofer lines A and B, and extending just beyond line B, with a mid-point in the range of $710 \pm m\mu$, and (II) between lines C and D with a mid-point in the range of $625 \pm m\mu$, which were in different positions from, or of different breadths than, the absorption bands of corn oil and linseed oil. In fact, the latter registered three absorption bands.

Other constants reported were: saponification value 188.8; iodine number (Hübl method) 115.6. They found 7.53 per cent of glycerol, 2.0 per cent of lecithin, 2.5 per cent of "paracholesterol" by the modified Bömer method, and 2.0 per cent by the elaidin reaction.

¹¹ *Z. ges. Getreide-, Mühlen- u. Bäckereiw.* **22**, 119 (1935).

¹² "Le blé Roumain," 581 pp., Bucharest, 1910.

¹³ *Biochem. Z.*, **177**, 168 (1926).

¹⁴ Private communication, July, 1941.

¹⁵ *J. Am. Chem. Soc.*, **21**, 758 (1899).

Germ oil was found by Balland¹⁶ to be composed of approximately 83.34 parts of fluid fats and 16.66 parts of solid fatty acids.

The constants of wheat-germ oil recovered by Alpers¹⁷ are included in Table 60. He noted that the lecithin content of the crude oil varied with the method of extraction, about 2.7 per cent being present in some of his preparations.

Substantial differences in the properties of the oils extracted from wheat germ and wheat endosperm (flour) are evident from the data of Ball,¹⁸ a portion of which is included in Table 60. One of the greatest differences was in the saponification number, which was 184.13 for the germ oil and 160.86 for the flour oil. The latter agreed fairly well with the data of Spaeth, but was substantially lower than de Negri's value. Iodine numbers of the flour oil are lower than those of the germ oil. Besides the data in Table 60, Ball found the mean molecular weight of the solid acids to be 245.08, and of the liquid acids 276.85. The saturated acids of the embryo oil constituted about 15.8 per cent of the fatty acids present. Oil separated by pressure from the germ had a slightly higher iodine number than that extracted by ether. Material with a melting point of 137.5°, which appeared to be sitosterol, was separated from flour oil.

Oil that had been extracted with ether from a fine sample of wheat germ was subjected to detailed study by Jamieson and Baughman²¹ with the results indicated in Table 62. From their data they computed the fatty acid content of the glycerides as follows:

Linolenic acid	10.0
Linolic acid	40.9
Oleic acid	27.8
Palmitic acid	12.8
Stearic acid	0.9
Lignoceric acid	0.3

An extensive study of the iodine and acid numbers of the crude fat extracted from soft winter wheats by petroleum ether was reported by Gustafson.²² In a collection of 89 commercial samples of the 1929, 1930, and 1931 crops received from mills, the iodine number ranged from 113.7 to 119.7, and the averages for the three crops were:

1929	115.2
1930	116.9
1931	117.0

The variability was small, and the differences between the averages of the three crops were of relatively small magnitude.

¹⁶ *Compt. rend.*, 137, 724 (1903).

¹⁷ *Chem. Ztg.*, 42, 87 (1918).

¹⁸ *Cereal Chem.*, 3, 19 (1926).

²¹ *Oil and Soap*, 8, 136 (1932).

²² *Cereal Chem.*, 9, 595 (1932).

Table 60. Fat Contents Reported By Several Investigators.

	Wheat embryo				Wheat flour		
	De Negri 1	Frankforter and Harding 2	Alpers 17	Ball 18	Spaeth 19	De Negri, Fabris, and Plucker 20	Ball 18
Specific gravity	0.9245 (15°/15°)	0.9292 (15°/15°)	0.9320 (25°/25°)	0.92485 (25°/1°)	0.9068 (15°/15°)	—	0.9673-0.9714
Refractive index	1.4750	1.48325 (20°)	1.4766	1.4686	1.4851 (25°)	1.4851	1.4714
Saponification No.	182.81	188.83	180.0	184.13	166.5	182.8	160.86
Iodine No.	115.17	115.64	122.6	123.32	101.5	96.1-112.5	105.43
Reichert-Meissel No.			0.75	0.475	2.8	2.95-4.95	
Hehner No.			95.31	93.71			
Polenske No.				0.25			2.51
Unsaponifiable (%)				3.51			
Melting pt. of solid acids (°C)	39.5						
Iodine No. of insol. acids	123.2			128.1			
Iodine No. of liquid acids				146.0			

19 *Z. angew. Chem.*, 1894, 294.

20 Quoted by Lewkowits, "Chemical Technology and Analysis of Oils, Fats, and Waxes," 5th ed., Vol. 2, p. 240, London, 1915.

The same constant was determined in the instance of 81 samples of pure varieties of the 1930 and 1931 crops received from the Indiana, Illinois, Michigan, Missouri, Ohio, Pennsylvania and Virginia Agricultural Experiment Stations. The range was from 114.0 to 119.2, or somewhat less than in the instance of the commercial wheat samples. The average iodine numbers for the two crops were:

1930	117.1
1931	116.8

Thus the variability of the pure variety samples was somewhat less than among the commercial soft winter wheats.

The acid number, as determined by Coleman's method, ranged from 6.28 to 23.25 among the commercial wheat fats, and from 5.12 to 18.57 among the pure variety samples of wheat. The averages were:

	Commercial wheats	Pure varieties
1929 crop	11.01
1930 crop	12.76	9.68
1931 crop	9.88	11.77

It is evident that this property of the fat is much more variable than the iodine number; this is not surprising in view of the ease with which it can be modified or increased with the lapse of time in storage, or by the development of unsoundness in the grain. When the wheat meal was allowed to stand for 2½ months, the acid number increased two- to six-fold in a series of 24 samples, while the iodine number decreased very slightly, and by the order of 2 to 8 units at the same time.

Fats extracted by ethyl ether from flour, bran, and germ, and dried in a desiccator for 48 hours, were studied by Herd and Amos.²³ As shown in the following table, there is less difference between the bran and flour fats as far as the constants involved are concerned than between the flour and germ fats:

Source of fat	Refractive index, N_D^{20}	Bromine value	Iodine value
Flour	1.4830	67.0	106.40
Bran	1.4800	70.0	111.16
Germ	1.4790	81.3	129.10

The bromine values were determined by Toms' method, and the iodine values were calculated from them.

Acid hydrolysis and extraction resulted in a larger return of fat from flour, bran and germ than direct extraction with either ethyl or petroleum ether. Moreover, the fats extracted by the acid hydrolysis method did

²³ *Cereal Chem.*, 7, 251 (1930).

not contain significantly more nitrogen or phosphorus than those recovered by direct extraction. When an alcoholic-ether reagent was used as the solvent, the nitrogen and phosphorus content of the extracted lipoids was substantially greater, which suggested the presence of substances other than simple fatty-acid glycerides. Thus the percentages of phosphorus in the fats extracted by (I) ethyl ether alone, and (II) 70 per cent ethanol plus ethyl ether in three mill products were as follows: patent flour (I) 0.27, (II) 1.07; bran (I) 0.11, (II) 0.34; germ (I) 0.28, (II) 0.48. Somewhat similar differences appeared in the instance of the nitrogen content of the same fat preparations.

The constants of materials extracted by acetone from patent flour milled from *vulgare* (var. Marquis) and *durum* (var. Mindum) were reported by Walde and Mangels²⁴ as follows:

	Acetone extracts of patent flour	
	<i>Vulgare</i> (Marquis)	<i>Durum</i> (Mindum)
Acetone-soluble material (%)	1.25	1.26
Nitrogen in acetone extract (%)	0.113	0.110
Phosphorus in acetone extract (%)	0.090	0.090
Iodine number	92.2	109.3
Saponification number	133.5	159.1
Unsaponifiable matter (%)	4.37	6.01
Ester number	113.4	141.3
Free acid number	20.11	17.78

On comparing these constants with the values recorded in Tables 61 and 62 it is evident that the acetone extracted lipid substances from the *vulgare* flour with a lower iodine number and saponification number than the lipids extracted with the conventional solvents. The acetone-soluble lipid of the *durum* wheat flour is very different from that of *vulgare* wheat flour in several particulars.

The ether-soluble constituents of hard spring (Marquis) wheat, *durum* (Kubanka), and a white (Federation) wheat flours were extracted by Martin and Whitecomb.²⁵ Certain of the constants and properties of these ether-soluble constituents were determined by them with the results recorded in Table 61.

There is evidence here of some differences among the crude fats recovered from these three sources. The *durum* flour fat was lowest in coefficient of viscosity, solution temperature of suspended solids, apparent molecular weight, optical rotation, and acetyl number, and highest in iodine number, Reichert-Meissl number, and coefficient of viscosity. From these few determinations it may be improper to generalize concerning

²⁴ *Cereal Chem.*, 7, 480 (1930).

²⁵ *Cereal Chem.*, 9, 275 (1932).

genetic differences, but the data suggest that such may exist between different species of the genus *Triticum*.

Table 61. Physical and Chemical Properties of Ether-Soluble Constituents Extracted from Hard Spring (Marquis), *Durum* (Kubanka) and White (Federation) Wheat Flours, as Reported by Martin and Whitcomb.²⁵

Properties of ether-soluble constituents	Marquis	Kubanka	Federation
Non-volatile ether-soluble constituents extracted from 1 kg flour (grams)	13.29	14.87	11.71
Color value (the color of a solution of 0.005 g $K_2Cr_2O_7$ in 100 cc taken as 1.0)	7.0	61.0	33.0
Coefficient of viscosity	1.47	0.49	1.42
Specific gravity	0.9399	0.9251	0.9418
Surface tension (dynes per cm)	36.57	35.91	36.68
Solution temperature of suspended solids ($^{\circ}C$)*	45 to 49	-16 to -18	41 to 45
Index of refraction (angular degrees)	1.4805	1.4785	1.4798
Optical rotation (angular degrees)	0.69	0.48	0.55
Freezing-point depression of 1.0 g in 10 cc benzine ($^{\circ}C$)	0.838	0.851	0.618
Apparent molecular weight	695	689	942
Saponification number (Koettstorfer number)	182.2	177.7	183.4
Iodine number	121.9	127.6	126.8
Acetyl number	21.8	18.4	24.2
Reichert-Meissl number	1.50	1.56	1.06
Total acids (equivalents per kg of oil)	2.7744	2.9037	2.6916
Water-soluble acids (equivalents per kg of oil)	0.2536	0.3671	0.1868
Water-soluble acids (equivalents %)	9.1	12.6	6.9
Water-insoluble acids (equivalents per kg of oil)	2.5208	2.5366	2.5048
Water-insoluble acids (equivalents %)	90.9	87.4	93.1
Nitrogen (%)	0.57	0.61	0.30
Phosphorus (%)	0.15	0.16	0.14

* Temperature at which suspended crystals (sterols) dissolved in oil.

Wheat-germ fat was reported by Hilditch²⁶ to contain the following percentages of fatty acids:

Palmitic	13.8%
Stearic	1.0
Oleic	30.0
Linolic	44.1
Linolenic	10.8

The lipid obtained by alcohol-ether extraction of wheat germ was examined by Sullivan and Bailey.²⁷ A total of 13.68 per cent of lipid was obtained from the germ (11.2 per cent moisture), and this lipid contained 0.415 per cent nitrogen and 1.23 per cent phosphorus. The physical and chemical characteristics of this lipid are recorded in Table 62. Based upon these data, the total unsaturated fatty acids, amounting to 84.0 per cent, were estimated to be comprised of the following:

²⁶ *Chemistry & Industry*, **54**, 139 (1935).

²⁷ *J. Am. Chem. Soc.*, **58**, 283 (1936).

α -Linolenic acid	1.83
β -Linolenic acid	1.72
α -Linolic acid	22.32
β -Linolic acid	29.99
Oleic acid (by difference)	28.14
Total	84.00

The saturated acid fraction was largely palmitic acid, in the range of 73.5 per cent of the total acids, with about 19 per cent of stearic and 7.5 per cent of lignoceric acids. This is a larger proportion of stearic and lignoceric acids than was reported by Jamieson and Baughman.²¹

A carbohydrate was also encountered in this alcohol-ether extract of wheat germ that was not completely identified. While this fraction gave some reduction when tested by the Bertrand method, the reducing power was increased by inversion. Glucosazone was prepared from the inverted product. When it was acetylated, the physical properties of the deriva-

Table 62. Physical and Chemical Characteristics of the Extracts of Wheat Flour and Wheat Germ.

	Ether extract of germ Jamieson and Baughman ²¹	Alcohol-ether extract of germ Sullivan and Bailey ²⁷	Petroleum ether extract of flour Sullivan and Howe ²⁸
Specific gravity	0.9268 (25°/25°)	0.9326 (26°/26°)	0.9542 (26°/26°)
Refractive index	1.4762 (20°)	1.4800 (20°)	1.4824
Acid value	7.6	6.95	21.6
Saponification value	186.5	184.0	177.8
Iodine No.	125.6 (Hanus)	125.0 (Rosenmund)	
Thiocyanogen No.	79.7	84.7	
Hexabromide No.	Trace	2.28	
Acetyl value	9.9	16.7	47.7
Reichert-Meissl No.	0.2	0.77	7.9
Polenske No.	0.35	0.44	1.1
Hehner No.	...	89.0	87.0
Soluble acids as butyric	...	1.44	5.4
Ester No.	...	177.05	156.2
Unsapnifiable matter (%)	4.70	4.00	5.48
Iodine No. of unsapon.	97.3	...	
Thiocyanogen No. of unsapon.	62.0	...	
Saturated acids (Twitchell) (%)	...	16.00	15.60
Saturated acids (Bertram) (%)	...	17.87	
Saturated acids (lead salt-ether) (%)	13.3 (corr.)	...	
Unsaturated acids (%)	75.3 (corr.)	84.00*	84.40†
Iodine No. of total fatty acids	...	129.9	125.0
Thiocyanogen No. of total fatty acids	...	79.3	79.9
Iodine No. of unsat. fatty acids	160.7	153.0	146.0
Mean mol. wt. of total fatty acids	...	278.0	276.4
Mean mol. wt. of sat. fatty acids	...	262.2	264.3
Mean mol. wt. of unsat. fatty acids	...	281.0	283.3

* Percentage of total fatty acids determined.

† By difference.

tive did not match those of the acetylated sugars described in the literature.

The petroleum-ether extract of wheat flour amounting to 1.38 per cent (dry basis) of the flour was found by Sullivan and Howe²⁸ to differ from the wheat-germ lipids. As was true of Ball's¹⁸ earlier comparisons, the flour lipids exhibited less unsaturation. Less linolic acid and more oleic acid was found, while the percentage of total saturated acids was about the same in the two lipid preparations. More volatile soluble fatty acids in the wheat-germ extract are demonstrated by the decidedly higher Reichert-Meissl number.

The percentages of the fatty acids were computed from the lipid constants of the petroleum ether extract of flour with the following results:

Total saturated acids (83% palmitic)	15.6%
α -Linolenic acid	0.84
β -Linolenic acid	2.96
α -Linolic acid	25.49
β -Linolic acid	20.51
Oleic acid (by difference)	34.60

Whereas about 70 per cent of the unsaponifiable material from wheat germ was precipitated by digitonin, only about half that of the flour lipids was precipitated by that reagent. It was assumed that most of the combined sterols of flour occur as sitosterol palmitate.

Table 63. Various Constants of the Petroleum-ether Extract of Bran, Low-grade Flours ("Bottoms") and Patent Flour, as Reported by Barton-Wright.²⁹

Constant	Bran oil	1½% bottom flour oil	12½% bottom flour oil	Patent flour oil	Germ oil
Acid value*	87.47	26.81	26.22	19.25	24.57
Saponification value	182.1	175.6	172.6	164.1	184.0
Iodine value	127.1	116.9	114.9	106.9	127.4
Saturated acids† (%)	16.0	16.5	16.1	16.6	16.0
Unsaturated acids† (%)	84.0	83.5	83.9	83.4	84.0
Iodine value of mixed acids	134.5	132.7	131.6	133.1	134.1
Iodine value of unsaturated acids	152.4	153.3	153.1	157.8	160.0
Mol. wt. of mixed acids	315.0	292.0	306.0	299.0	278.0
Mol. wt. of saturated acids	279.0	267.0	266.0	267.0	266.0
Mol. wt. of unsaturated acids	358.0	303.0	346.0	334.0	309.0
Unsaponifiable residue	12.1	4.1	4.1	5.6	

* Extraction of the bran oil made 4 months after milling and of the flour oils 1 month after milling.

† Expressed as percentage of the total mixed acids.

The oil recovered from germ, bran, low-grade flour ("bottom"), and patent flour by extraction with petroleum ether was subjected to fairly detailed study by Barton-Wright,²⁹ with the results indicated in part in Table 63. No large differences in the observed constants were apparent

²⁸ *Cereal Chem.*, 15, 716 (1938).

²⁹ *Cereal Chem.*, 15, 723 (1938).

except in the instance of the iodine value. This exhibited a tendency to increase in progressing from the refined or patent flour, through the lower grades, to the bran and germ. The unsaponifiable residue of the bran oil was also greater than that of the flours. The lack of agreement in the acid values may be due in part to the fact that the extraction of the flours was made 1 month after milling, and that of the bran oil 3 months later.

Using the iodine number of the fatty acids of the germ oil (= 134.1), the thiocyanogen number (= 80.27) and 16.0 per cent of saturated fatty acids, in the equation of Kaufmann and Keller, the following percentages of unsaturated fatty acids were estimated to be present:

Oleic acid	25.2%
Linolic acid	53.5
Linolenic acid	5.13

In a previous communication Barton-Wright³⁰ assumed the presence in germ oil of another unsaturated fatty acid which is probably palm-itoic acid, with one double bond in the molecule. This acid had not been sufficiently characterized to make possible its complete identification, and if present, it is included with the oleic acid in the foregoing estimate.

To the petroleum-ether solutions of the flour, germ and bran fats, Barton-Wright added four times their volume of acetone; after they had stood overnight, he recovered a mixture of compound lipids including phosphatides and glycolipids as shown in Table 64. The relative propor-

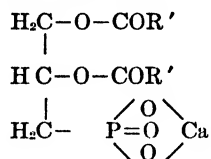
Table 64. Petroleum-ether Extract of Germ, Bran, Low-grades ("Bottoms") and Patent Flour, Including the Acetone-insoluble Fraction, as Reported by Barton-Wright.²⁹

Product	Total petroleum ether extract (%)	Acetone- insoluble fraction (%)	Proportion of total fat in- soluble in acetone (%)
Germ	6.72	0.434	6.5
Bran	3.03	0.276	9.1
Bottom 1½% flour	2.84	0.214	7.5
Bottom 12½% flour	1.55	0.187	12.1
Patent flour	0.91	0.183	20.3

tion of the crude fat so precipitated was quite variable, ranging from 20.3 per cent in the patent flour fat to 6.5 per cent in the germ fat. This precipitate was further purified by dissolving in anhydrous ether and reprecipitating with acetone (4 volumes). A small amount of kephalin was identified in consequence of the recovery of β -amino-ethyl alcohol chloroaurate which had a M. P. = 189°. Choline was recovered as the choline mercuric chloride, M. P. 254°, and further confirmation of its

³⁰ *Cereal Chem.*, 15, 521 (1938).

presence was afforded by the reaction with cadmium chloride. This indicated the presence of lecithin, as well as kephalin, but the amounts were small, and the main substance appeared to be a magnesium salt of the nitrogen-free lipid, phosphatidic acid, previously identified by Chibnall and Cannon ³¹ as a calcium salt having the structural formula:



The acetone-insoluble fraction from the bran oil appeared to contain larger proportions of lecithin and kephalin; from this, Barton-Wright ²⁹ reasoned that these substances are more characteristic of the petroleum-ether extract of the envelope of the grain, whereas in the remainder of the grain the magnesium salt of phosphatidic acid is more prominently represented in this fraction.

When the residue from extraction with petroleum ether was re-extracted with a mixture of 80 parts of benzine and 20 parts of alcohol, Barton-Wright recovered appreciable quantities of lipoids; the average of duplicated determinations gave the following percentages: germ, 2.26; bran, 3.10; bottom 1½ per cent flour, 0.91; bottom 12½ per cent flour, 0.76; patent flour, 0.72. Although a detailed examination of this material was not reported, it seems probable that phospholipids would be strongly represented.

A petroleum-ether extract of fresh patent flour, when emulsified in water, was found by Balls and Hale ³² to give a positive nitroprusside test, indicating the presence of substances having sulfhydryl groups. When recourse was had to a process of fractionation or purification, a preparation was recovered which contained 2.94 per cent of sulfur, and 13.4 per cent of total nitrogen. In many particulars this material did not comport itself like an ordinary protein, however, although it resembled a protein derivative. The question was raised as to whether it exists originally in combination with a lipid or is merely soluble in lipid-containing fat solvents.

SECTION III: PHOSPHOLIPIDS

One of the earliest studies of the phospholipids of wheat was that of Töpler's, ³³ who reported the presence of 0.25-28 per cent of phosphorus in the oil.

³¹ *Biochem. J.*, 21, 233 (1927).

³² *Cereal Chem.*, 17, 243 (1940).

³³ *Jahresber. Agr. Chem.*, 4, 57 (1861).

Lecithin content of wheat, as determined by ether-alcohol extraction, was found by Schulze and Steiger³⁴ to be 0.65 per cent. The phosphorus content of the extract was 0.025 per cent. Rye contained somewhat less, and barley somewhat more lecithin than wheat, the content being 0.57 and 0.74 per cent, respectively.

Using the phosphorus content of those substances which were soluble in ether, followed by alcohol digestion at 60°, as the basis of calculation, and assuming 3.84 per cent of phosphorus in lecithin, Schulze and Frankfurt³⁵ found 0.65 per cent of lecithin in wheat, and 1.55 per cent in wheat bran. In general, the common cereals that were analyzed contained from 0.57 to 0.74 per cent of lecithin. Wheat germ analyzed by Frankfurt³⁶ was reported to contain 1.55 per cent of lecithin, which was identical with the earlier analysis of wheat bran. In the same germ they found 13.51 per cent of total fat, which was somewhat less than the 14.25 per cent reported by Haberlands in 1875. Cholesterin (cholesterol) was also identified in the germ, 0.44 per cent being found. In another paper from the Zurich laboratory, Schulze³⁷ discussed the details of the methods found to be most satisfactory in the extraction and quantitative estimation of lecithin.

The lecithin content of various plant products, including several cereal grains, was determined by Bitte.³⁸ After extracting with ether and methyl alcohol (hot) twenty times, a total of 0.495 per cent of lecithin was recovered from wheat, as compared with 0.65 per cent reported by Schulze and Steiger.

The method of extraction was then simplified to include only the treatment with methyl alcohol (heated) and an increased quantity of lecithin was then recovered from wheat, namely 0.578 per cent. On saponification and hydrolysis of the soap, phosphoric acid and trimethylamine as decomposition products of choline were recovered.

Incidental to a study of edible pastes, Beythien and Wrampelmeyer³⁹ analyzed twelve samples of semolinas. The total ash content averaged 0.71 per cent (all analyses reported on a dry basis), the total phosphoric acid 0.3165 per cent, and the lecithin-phosphoric acid 0.0165 per cent. It thus appeared that only about 5 per cent of the total phosphorus of these semolinas was present in the lecithin, as determined by these investigators. Jaeckle⁴⁰ also found about the same quantity of lecithin phos-

³⁴ *Z. Physiol. Chem.*, **13**, 386 (1892).

³⁵ *Landw. Ver. Sta.*, **43**, 307 (1894).

³⁶ *Ibid.*, **47**, 449 (1896).

³⁷ *Ibid.*, **49**, 203 (1898).

³⁸ *Z. physiol. Chem.*, **19**, 488 (1894).

³⁹ *Z. Untersuch. Nahr. u. Genussm.*, **4**, 145 (1901).

⁴⁰ *Ibid.*, **7**, 513 (1904).

phorus in the sample of wheat flour which he analyzed, namely 0.0152 per cent, reported as "alcohol-soluble phosphoric acid (P_2O_5)."

Using the method of Schulze *et al.* for the preparation of phosphatides, Winterstein and Hiestand⁴¹ treated wheat flour with ether, followed by 95 per cent alcohol (hot). The alcohol was removed by distillation and the phosphatide in the residue was taken up with ether. The brown, salvy, hygroscopic mass which was recovered on vaporizing the ether contained 0.80 per cent phosphorus by one method, although the Grete method indicated 0.86 per cent of phosphorus. After several attempts at purification, a lecithin preparation was obtained which was apparently freer from sterols than the first crude product, and contained 1.5 per cent of phosphorus; this may have been as high as 2.6 per cent.

Furfural appeared on heating with 12 per cent HCl. The nitrogen content of 0.74-1.6 per cent that was found in the purest preparations was higher than the 0.64 per cent calculated for pure lecithin. A sugar, apparently *d*-glucose, in the amount of about 16 per cent was recovered, but the authors were apparently uncertain as to whether (a) it was present as a monosaccharide in the original preparation or resulted from the hydrolysis of a di- or polysaccharide, and (b) whether it occurred in organic combination in the lecithin molecule. A pentose in the amount of about 0.5 per cent was also indicated. They concluded that a nitrogenous base other than choline was present, since nitrogenous compounds were encountered which, like neurine, were not precipitated by phosphotungstic acid.

In an extension of this study by Winterstein and Smolenski,⁴² the phosphatide preparation recovered by treating wheat flour with alcohol proved to be a mixture containing cholesterol, its ester, free fatty acids, and small quantities of other compounds. On cleavage, a nitrogenous base which appeared to be trigonellin was recovered.

In the phosphatides prepared from wheat germ by Smolenski,⁴³ the phosphorus content ranged from 3.51 to 3.9 per cent. Fractionation with acetone resulted in the recovery of an acetone-soluble crystalline substance, melting at 82-83°, and containing 5.48 per cent of phosphorus. An oily mixture was also recovered from the acetone-soluble material which was chiefly composed of fats and fatty acids.

The formula of wheat lecithin depicted by Kosutany⁴⁴ was that of a di-steryl ester.

Free lecithin constituted 0.142 per cent of the wheat kernel, and combined lecithin 0.297 per cent, in the analyses reported by Bernardini and

⁴¹ *Z. physiol. Chem.*, 54, 288 (1908).

⁴² *Z. physiol. Chem.*, 58, 506 (1909).

⁴³ *Ibid.*, 58, 522 (1909).

⁴⁴ "Der ungarische Weizen und das ungarische Mehl," Budapest, 1907.

Chiarulli.⁴⁵ The ratio of free to combined lecithin tended to decrease somewhat during germination of the grain.

Entire (undecorticated) wheat grains were found by Schulze⁴⁶ to contain 1.25 per cent of phosphatides, calculated from their phosphorus content. In the germ he found 13.51 per cent of crude fat, 1.55 per cent of lecithin, and 0.44 per cent of phytosterol.

Phosphatides were separated from wheat embryo by Channon and Foster,⁴⁷ and phosphatidic acid, lecithin and kephalin were identified. In terms of their phosphorus content the ratio was about 4:4:1 in the order named; at least 42 per cent of phosphatidic acid was present in the total phosphatide. This phosphatidic acid may represent an intermediate compound in the synthesis of lecithin and kephalin.

Glutens prepared from flours of lowest quality were found by Sullivan and Near^{48, 49} to have a higher lipid content than glutens from flours of superior quality. In a later and more extended study of flour lipoids they⁵⁰ emphasized that most of the lipoids are removed with hot alcohol, and consequently the following extraction with ether can be shortened to one-half hour. The resulting extract included not only phosphatides but also neutral fats, fatty acids if present, chlorophyll, and sterols. Accordingly, it is greater in quantity than a simple ether extract, although the ratio between the ether extract and the total lipoids is narrower in the lower-grade flours (clear and low-grade), bran, germ, and wheat than in the high-grade flours. Moreover, as detailed in the section of this monograph dealing with the minerals, the percentage of total phosphorus in the lipoids decreases with decreasing refinement.

In the data of Sullivan and Near it is also evident that both ether extract and lipid content of the flours increased with decreasing refinement. There appears to be a correlation between lipid and ash content of these flours. This is shown by the data in Table 65.

Table 65. Ether Extract and Lipoid Content of Wheat and Wheat Mill Products, as Reported by Sullivan and Near.⁵⁰

Material	Yield in per cent of wheat	Ash (%)	Ether Extract (%)	Lipoid (%)
Wheat	100	1.855	2.30	3.02
Patent flour	58	0.464	1.15	1.79
Clear flour	12	0.778	1.88	2.44
Low-grade flour	2.6	1.647	3.51	4.39
Bran		6.497	4.76	5.74
Germ	<1.	4.628	10.81	12.04

⁴⁵ *Staz. sper. agrar. ital.*, 42, 97 (1909).

⁴⁶ *Landw. Versuchs. Stat.*, 73, 85 (1910).

⁴⁷ *Biochem. J.*, 28, 853 (1934).

⁴⁸ *Ind. Eng. Chem.*, 19, 159 (1927).

⁴⁹ *Ibid.*, 19, 498 (1927).

⁵⁰ *Cereal Chem.*, 5, 163 (1928).

While the lipids extracted from wheat germ by alcohol-ether extraction, as conducted by Sullivan and Near,⁵¹ decreased somewhat on storage for three months in closed containers, namely from 15.24 per cent to 13.41 per cent, the phosphorus in the lipid extract decreased much more relatively, *i.e.*, from 0.518 per cent in the fresh germ lipid to 0.917 per cent in that stored for three months. The same was true of the bran lipids, and, to a lesser degree, of the wheat lipids, but was not the case in patent flour lipids. In general, the same trends were observed when ether, acetone, and ethyl acetate were used as lipid solvents, as far as phosphorus content was concerned. The total quantity of lipids extracted with these three solvents did not decrease on storage in a closed container, however.

When these products were stored in cloth bags under conditions such that they lost about one-third or more of their moisture in three months, the results were quite different; the percentage of phosphorus in the bran and germ lipids extracted with alcohol-ether did not decrease under these conditions. Evidently moisture is essential to the changes which effect the cleavage of phospholipids to non-soluble (alcohol-ether solvent) phosphorus-containing derivatives.

Table 66. Percentage of Lipid Extracted from Fresh Material by Various Solvents, as Shown by Sullivan and Near.⁵¹

Material and solvent	Extract (%)	Phosphorus in extract (%)
<i>Germ</i>		
Alcohol-ether	15.24	0.518
Ether	12.05	0.114
Acetone	13.06	0.164
Ethyl acetate	13.16	0.268
<i>Bran</i>		
Alcohol-ether	7.36	0.464
Ether	5.60	0.091
Acetone	7.05	0.150
Ethyl acetate	7.26	0.248
<i>Wheat</i>		
Alcohol-ether	3.16	0.481
Ether	2.50	0.145
Acetone	3.19	0.188
Ethyl acetate	3.40	0.301
<i>Patent flour</i>		
Alcohol-ether	1.29	0.575
Ether	1.13	0.234
Acetone	1.25	0.433
Ethyl acetate	1.20	0.302

Referring further to the fresh material, the phosphorus content of the lipids extracted from the several materials by the four solvents was quite different, as shown by the examples in Table 66.

⁵¹ *Ind. Eng. Chem.*, **25**, 100 (1933).

The percentage of lecithin in various grades of flour varied less in relative terms than the percentage of total lipids in the studies conducted by Geoffroy,⁵² as shown by his data in Table 67. The unsaponifiable matter mounted appreciably in progressing from the flours to the bran and germ. As usual, the germ was characterized by a high content of total lipids, and a lecithin content about five times as high as the flour.

Table 67. Lipids of Flour and Other Mill Products, as Reported by Geoffroy.⁵²

Material	Lipid extract (%)	Fatty material (%)	Saponification number	Lecithin (%)	Unsaponifiable (%)
Flour from center of grain	1.18	0.84	157	0.23	1.9
Flour from median zone	1.48	1.26	156	0.23	1.9
Flour from cortical zone	2.36	1.64	171	0.30	1.6
Bran	3.25	2.60	149	0.50	2.8
Germ	14.14	12.00	169	1.25	3.2

In the opinion of Nottbohm and Mayer,⁵³ the cholin of flour was not present as the free base, but only as cholin-lecithin. The phosphatide content of six flour samples analyzed by them ranged from 1.17 to 1.56 per cent, with an average of 1.35 per cent. The grits ("griess") or middlings contained somewhat more, averaging 1.80 per cent.

Storage of flours in sacks appeared to result in certain instances in reducing the quantity of the substance extractable by ether I, and by alcohol-ether II (1:1 mixture) in the studies of Sinclair and McCalla.⁵⁴ The average percentage in I in the instance of the fresh flours was 1.16 per cent, and in II was 1.53 per cent.

SECTION IV: STEROLS

Crystals which appeared to be identical with the "cholesterin" previously noted by Bencke in plant fat were recovered from the fat of wheat gluten by Ritthausen.⁵⁵ From 15 "pfund" of wheat flour only a few decigrams of these crystals was obtained, which was insufficient for detailed analysis. With Schiff's reagent, a violet to blue coloration was obtained.

A phytosterol recovered by Burian⁵⁶ from an acetone extract of wheat germ was named "sitosterin" (Eng. "sitosterol") from *sitos* (Gr. wheat, cereal), with a melting point of 137.5° (uncorr.) as compared with 145° (Hesse) or 148.5° (corr., Reinitzer) for cholesterol from gall stones. Hesse's phytosterol had been reported as having a melting point of 132.5°.

Like cholesterol, Burian's sitosterol gave the Liebermann-Burchard, the Hesse-Salkowski, and the Schiff (with HNO₃) reactions.

⁵² *Bull. soc. botan. France*, **81**, 17 (1934).

⁵³ *Z. Untersuch. Lebensm.*, **67**, 369 (1934).

⁵⁴ *Can. J. Research C.*, **15**, 187 (1937).

⁵⁵ *J. prakt. Chem.*, **88**, 145 (1863).

⁵⁶ *Monatsh.*, **18**, 551 (1897).

On acetylation, the melting point remained unaltered. It was levorotatory, with $(\alpha)_D = -26.71^\circ$, as contrasted with -29.92° for cholesterol from gall stones. Its empirical formula was computed to be $C_{27}H_{44}O + H_2O$. A bromide, acetate, propionate, benzoate, and chloride were prepared.

Another sterol, called parasitosterol, was apparently present in the mother liquor from which the sitosterol was recovered, with $(\alpha)_D = -20.8^\circ$, and a melting point of 127.5° (uncorr.). This was assumed to be isomeric with sitosterol.

An excellent review of the earlier studies of the sterols was presented by Ritter,⁵⁷ who confirmed Burian's⁵⁶ observation of the presence of sitosterol as a characteristic component of phytosterol from wheat germ.

From the entire wheat grain Ellis⁵⁸ recovered a phytosterol preparation from the unsaponifiable matter of the ether extract which appeared to be identical with Burian's and Ritter's sterols from wheat embryo. From its properties Ellis concluded that it was chiefly sitosterol. Bran sterols did not appear to be identical in their make-up with those from the entire grain. From wheat germ she recovered about 0.5 per cent of sterols, which, however, were not fully described.

Studies of maize oil conducted by Gill and Tufts⁵⁹ convinced them that the principal sterol present is not cholesterol, but is identical with Burian's "sitosterol" and with Reinitzer's⁶⁰ "hydrocarotin".

Corn oil yielded a phytosterol which comprised most of the unsaponifiable matter, and was identical with sitosterol, in the studies made by Anderson and Moore.⁶¹ Its melting point was 137.5° , and the specific rotation in chloroform solution was $(\alpha)_D^{20} = -34.38^\circ$. The acetate melted at 127° -. No stigmasterol could be identified in the phytosterol.

These investigators recovered 2.01 per cent of unsaponifiable matter from crude corn oil and 1.68 per cent from refined edible corn oil, indicating that the process of refining resulted in removing some of the unsaponifiable material.

On extending these studies to the endosperm of corn, Anderson⁶² recovered two fractions of phytosterol from the unsaponifiable matter of the ether extract which differed in composition and properties. Rather large proportions of ordinary sitosterol were encountered, such as were described in the preceding paragraph, mixed with optically active dihydro-sitosterol, $C_{27}H_{47}OH \cdot H_2O$, M. P. $138-139^\circ$, $(\alpha)_D^{20}$ (in chloroform solution)

⁵⁷ *Z. physiol. Chem.*, **34**, 430 (1902); **34**, 461 (1902).

⁵⁸ *Biochem. J.*, **12**, 160 (1918).

⁵⁹ *J. Am. Chem. Soc.*, **25**, 251 (1903).

⁶⁰ *Monatsh.*, **7**, 597 (1886).

⁶¹ *J. Am. Chem. Soc.*, **45**, 1944 (1923).

⁶² *J. Am. Chem. Soc.*, **46**, 1450 (1924).

+25°. The acetyl derivative in the same solvent has an $(\alpha)_D^{20} = +14.41^\circ$, M. P. 138°. Dihydrositosterol was also found in corn bran.

In addition, a brownish-yellow substance was recovered from the unsaponifiable, which was not examined further.

Dihydrositosterol as thus obtained crystallized in the same form as sitosterol, but the crystals were larger and denser. It did not give the Liebermann-Burchard reaction, as did the sitosterol, and the Whitby reactions were atypical. It did not absorb bromine.

Unsaponifiable matter from the fat extracted from wheat endosperm by Anderson and Nabenhauer⁶³ also contained an unsaturated sterol identical with that recovered from corn, which is described above, and a saturated sterol corresponding to dihydrositosterol, M. P. 144-145°, $(\alpha)_D^{20} + 25.82^\circ$:

Extraction of wheat bran with petroleum ether gave 3 per cent of fat which contained 6 per cent of unsaponifiable matter. A total of 38 crystallizations from petroleum ether resulted in a progressive modification of the properties of the resulting phytosterol crystals, with increasing M. P. reaching 144-145°; and an $(\alpha)_D^{20}$ which changed from -4.80° after the fifth recrystallization to $+25.82^\circ$ after the 38th recrystallization. Anderson and Nabenhauer believe this dihydrositosterol to be identical with the saturated sterol that occurs in corn endosperm.

Phytosterols of wheat germ oil were further studied by Anderson, Shriner and Burr,⁶⁴ and were found to constitute a mixture of dihydrositosterol, and at least three sterols isomeric with sitosterol, designated by them as α -, β -, and γ -sitosterol. The α - and β - forms were not obtained in a pure state, but the γ -sitosterol, which was the least soluble, was recovered in a fairly pure state, and its properties determined as follows: M. P. 147-148°, $(\alpha)_D$ about -42° ; acetyl derivative, M. P. 143-144°, $(\alpha)_D$ about -45° . It was also noted that the α -sitosterol differed from the other two in that it yielded a bromine substitution product that could not be debrominated.

On extending this study Anderson, Nabenhauer and Shriner⁶⁵ gave particular attention to the dihydrositosterol in plant fats. The crystalline sterols of wheat-germ oil that were examined appeared to contain not less than 1.6 per cent of dihydrositosterol. This was a minimum value, since considerable losses may have occurred during its isolation.

From the residue remaining after the saponification of wheat-germ oil, Power and Salway⁶⁶ recovered a substance which they assumed to be

⁶³ *Ibid.*, 46, 1717 (1924).

⁶⁴ *J. Am. Chem. Soc.*, 48, 2987 (1926).

⁶⁵ *J. Biol. Chem.*, 71, 389 (1927).

⁶⁶ *Pharm. J.*, 91, 117 (1913).

sitosterol. It was extracted with ethyl acetate, had a melting point of 138° , and the formula $C_{27}H_{46}O$. The fatty acids of wheat-germ oil included solid acids, with stearic and palmitic in about equal proportions, together with liquid acids which appeared to be mainly linolic.

The Salkowski test for sterols, involving the appearance of a red color when the chloroform solution is shaken with strong H_2SO_4 , and the Burchardt-Liebermann test, in which a violet coloration appears when the chloroform solution (2 cc) is treated with 20 drops of acetic anhydride and one drop of concentrated H_2SO_4 , are described by Ellis.⁶⁷

The chief phytosterol of the wheat grain was found to be the same as that in the embryo, sitosterol. A phytosterol was found in the bran which melted at 142° , and accordingly appeared to be different from the sitosterol of the grain and germ which melted at 137.5° . Moreover, the acetates had different melting points, that of the whole-grain phytosterol at 127° , and of the bran phytosterol at 137° . More phytosterol could be recovered from the embryo than from other tissues of the plant, although it was widely distributed. A recovery of 0.5 per cent from the germ, and 0.031 per cent from the entire grain was reported by Ellis.

A phytosterol which melts at 93° was separated from common wheat by Mangels,⁶⁸ which, on boiling for 6 to 8 hours with alcoholic potash, was converted into normal sitosterol (M. P. 137°). On similar treatment of *durum* wheat, only the normal sitosterol was recovered.

A phytosterolin was recovered from an ether extract of wheat germ by Nakamura and Ichiba⁶⁹ in the amount of 0.2 per cent of the germ. It appeared to be identical with ipuranol, a glucoside of phytosterol, previously recognized in other materials. Later Ichiba⁷⁰ concluded that the phytosterolin was a mixture of the glucosides of β - and γ -sitosterol.

The quantitative aspect of total sterol content of cereals was developed by Keding,⁷¹ who observed that additional sterols could be recovered from the ether-extracted residue by refluxing with water, followed by 20 per cent HCl, and then shaking out with three portions of ether. Thus in one series of three wheat extracts the average content of sterol was (I) 65.62 mg/% by direct ether extraction, and (II) 70.75 mg/% after hydrolysis. With a like number of wheat flour samples the yields were: (I) 99.0 mg/%, and (II) 115.0 mg/% in the two extracts, respectively.

On fractionating the acetyl derivatives of the phytosterol of wheat-germ oil, Ichiba⁷² identified dihydrositosterol and 4 isomeric sitosterols: α -sitosterol, M. P. $134-5^{\circ}$, and its acetyl derivative, M. P. $116-7^{\circ}$ [$(\alpha)_D$

⁶⁷ *Biochem. J.*, **12**, 160 (1918).

⁶⁸ *No. Dak. Agr. Exp. Sta. Bull.* **253**, p. 55 (1930).

⁶⁹ *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **15**, 137 (1931).

⁷⁰ *Ibid.*, **28**, 124 (1935).

⁷¹ *Biochem. Z.*, **254**, 374 (1932).

⁷² *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **28**, 112 (1935).

-23.6°]; β -sitosterol, the acetyl derivative having a M. P. of 136-7° [$(\alpha)_D = -31.5^\circ$]; γ -sitosterol, the acetyl derivative having a M. P. of 143°, [$(\alpha)_D = -47.7^\circ$]; δ -sitosterol, the acetyl derivative having a M. P. of 113.5-114.5°, [$(\alpha)_D = -24.4^\circ$]. An unidentified hydrocarbon was present in the mother liquors from which these sterols were recovered.

A sitosterol ester was recovered by Spielman⁷³ from the acetone extract of an unbleached patent flour. Its melting point was 93-94°; the sterol part isolated after saponification melted at 136.5° and exhibited a rotation of $\alpha_D = -13.3^\circ$. This weak levorotation suggested a mixture of sitosterols, rich in the α - and β -forms, and Spielman estimated this portion to represent 72 per cent of the sterols present. Dihydrosterol, which is dextrorotatory, with $(\alpha)_D$ variously reported from 25 to 35°, was estimated to constitute 28 per cent of the sterols.

The acid portion of the ester, as recovered after saponification with alcoholic alkali, was found to be palmitic acid.

The unsaponifiable fraction of their alcohol-ether extract of wheat germ was further examined by Sullivan and Bailey.⁷⁴ As indicated in Table 62, 4 per cent of this lipid preparation was unsaponifiable, of which about 70 per cent is a mixture of sterols, and the remainder was a viscous yellow oil. The latter is a rich source of Vitamin E. No paraffin, aldehyde or ketone could be detected. It was conjectured that unsaturated hydrocarbons of the polyene, with conjugated double bonds and no functional groups, are prominent in this yellow oil.

Of the sterol fraction, 56 per cent was in the free state. An unsaturated sterol with at least two double bonds was present, in addition to the isomeric sitosterols and dihydrositosterol. No addition product of ergosterol with maleic anhydride could be detected when the Windaus and Luttringhaus⁷⁵ method was applied to these sterols.

The sterols in domestic French wheat amounted to 0.069 per cent of the grain on the dry-matter basis as determined by Hagemann,⁷⁶ which was 4.6 per cent of the total fatty material present. *Durum* wheat grown in Tunis contained only 0.042 per cent of sterols, which was 2.9 per cent of the total fats. In wheat flour 0.038 to 0.048 per cent of sterols were found, which is about two-thirds of the level in the domestic wheat. The average percentage of the sterols in the total fat was 2.8 per cent in the instance of four flours that were analyzed.

A qualitative test which he devised for ergosterol gave negative results at the hands of Brückner⁷⁷ when applied to the mixed sterols from wheat embryo.

⁷³ *Cereal Chem.*, 10, 239 (1933).

⁷⁴ *J. Am. Chem. Soc.*, 58, 890 (1936).

⁷⁵ *Ber.*, 64, 850 (1931).

⁷⁶ *Bull. anc. élèves d'école Franc. de Meun.*, 62, 218 (1937).

⁷⁷ *Biochem. Z.*, 270, 346 (1934).

Sitosterol palmitate was observed by Dangoumau⁷⁸ to precipitate spontaneously from the ether extract of flour. In a note in 1935 he⁷⁹ indicated the constant presence of ergosterol in flour, in a concentration of 70-90 μg per 100 g flour. On continuing these studies, he reported⁸⁰ the evidence of absorption bands at 2935, 2810, 2700 \AA after ultraviolet irradiation, which bands are characteristics of ergosterol A. A feeble band was also visible at 2620 \AA .

Based upon a spectographic method, Dangoumau⁸⁰ estimated the quantities of ergosterol present in 100 g of various wheat products as follows:

Commercial germ	1.2 mg
Bread flour	0.082 mg
Primary reduction flour	0.046 mg
Low-grade flour	0.283 mg

A commercial wheat-germ preparation was extracted by Drummond, Singer and MacWalter⁸¹ with trichloroethylene, and a yield of 7.2 per cent of oil was obtained, which had the following characteristics:

Saponification value	182
Iodine No.	131
Refractive index (20°)	1.4773
Unsaponifiable matter (%)	5.76
Iodine No. of unsap.	97.6

While the greater part of the sterols consisted of sitosterol and dihydro-sitosterol, absorption bands characteristic of the triple system of ergosterol were observed upon spectroscopic examination. While it was deemed probable that certain of these absorption bands are related to ergosterol, it was conceded that other sterols may also be concerned.

The following substances were either isolated or detected in the unsaponifiable matter: sitosterol, dihydro-sitosterol, ergosterol (see foregoing paragraph), dihydroergosterol, lutein, kryptoxanthine, β -amyrin, squalene, another hydrocarbon of the squalene type, a liquid hydrocarbon which may have the composition $\text{C}_{18}\text{H}_{32}$, and a lipochrome which may be γ -carotene or rubixanthin.

A saturated C_{18} hydrocarbon constituted about 3 per cent of the wheat-germ oil analyzed by Drummond, Singer and MacWalter⁸²; the unsaturated hydrocarbon $\text{C}_{45}\text{H}_{76}$ was present to the extent of approximately 7 per cent.

⁷⁸ *Bull. soc. chim., biol.* 15, 1083 (1933).

⁷⁹ *Bull. soc. chim. (5)*, 2, 683 (1935).

⁸⁰ *Ibid.*, (5), 3, 988 (1936).

⁸¹ *Biochem. J.*, 29, 456 (1935).

⁸² *Biochem. J.*, 29, 2510 (1935).

Chapter 12

Minerals

GENERAL MINERAL ANALYSES

A compilation of the analyses of wheat ash published prior to 1871 was recorded by Wolff.¹ It is too extensive to be repeated here; moreover, as one scans the columns of Wolff's tabulation, it appears that some of the analyses may have been made by methods which would be regarded today as of doubtful accuracy. Thus there is a range of more than sixty-fold in the iron content of the ash of winter wheats, which is open to doubt. A range of about seven-fold appears in the calcium content of the ash of the same wheats, which again seems rather wide in the light of later investigations. Accordingly, these older data, while of general interest, may hardly be accepted in every instance as truly representative of wheat types as grown today in the same areas. Since the number of analyses is rather large, the averages may be of significance, however, and they are recorded in Table 68.

Table 68. Averages of the Mineral Analyses of Wheat Reported by Wolff.¹

Constituent	Winter wheat	Spring wheat
Total ash	1.97%	2.08%
	—Per cent of ash—	
KO	30.24	29.78
NaO	3.75	2.40
CaO	3.34	3.16
MgO	11.90	12.13
Fe ₂ O ₃	1.43	0.59
PO ₅	47.33	48.13
SO ₃	0.53	1.44
SiO ₂	2.19	1.56
Cl	0.48	0.50

The mineral content of the developing wheat kernel was followed by Heinrich² from July 4, a few days after ripening, until August 8, when it was ripe enough to harvest. The total mineral content was 4.33 per cent on the first date and 1.97 on the last. Percentages of constituents were: potassium 1.312 and 0.483; sodium 0.055 and 0.016; calcium 0.412 and 0.076; magnesium 0.514 and 0.250; iron oxide 0.024 and 0.059; phosphoric acid 1.740 and 1.019; chlorine 0.326 and 0.069; silica 0.021 and

¹ "Aschen-Analysen von landwirtschaftlichen Producten," Berlin, 1871.

² *Ann. Landw. Preuss. Staat.*, 57, 31 (1871).

0.039. Note that the iron content increased in percentage, as well as in total quantity, at the time that the other mineral elements (except SiO_2) were decreasing in percentage.

One of the early studies of the composition of the ash of various mill products was reported by Dempwolf.³ The results of his analyses, as shown in Table 69, indicate a fairly constant percentage of phosphorus and potassium in the ash, and of iron in the principal flour streams. The ratio of calcium tended to fall, and of magnesium to rise in progressing

Table 69. Percentage and Composition of the Ash of the Various mill Products, as Reported by Dempwolf.³

Grade and Mark	Percent- age of output	Total Ash (%)	Composition of Ash					
			Fe_2O_3 (%)	CaO (%)	MgO (%)	KO (%)	NaO (%)	PO_5 (%)
Kochgriese								
A	0.489	0.398	0.525	7.296	6.899	34.663	0.988	49.721
B		0.386	0.583	7.718	6.857	34.669	0.891	49.218
Auszugmehle								
0	3.144	0.380	0.630	8.057	7.008	35.482	0.744	48.896
1	2.635	0.416	0.643	7.946	7.105	35.285	0.675	48.976
2	5.291	0.452	0.627	7.454	7.795	34.254	0.678	48.519
3	7.165	0.481	0.635	7.092	8.343	33.876	0.690	49.306
Semmelmehle								
4	14.757	0.586	0.596	6.798	9.924	32.715	0.650	50.056
5	17.925	0.611	0.570	6.791	10.574	32.239	0.726	50.187
Brodmehele								
6	15.419	0.764	0.334	6.626	10.870	30.386	0.946	50.146
7	6.805	1.176	0.425	5.536	12.234	30.314	1.260	50.204
Schwarzmehele								
8	2.576	1.549	0.484	4.741	12.947	30.299	0.974	50.173
Kleien								
9	9.516	5.240	0.208	2.747	16.861	30.672	0.701	50.152
10	9.000	5.680	0.426	2.502	17.349	30.142	1.080	49.112
Kopfstaub								
11	1.290	2.648	1.671	8.203	13.023	31.489	2.144	44.054

from the more refined or low-ash to the less refined or high-ash flours. The ratio of calcium (as CaO) to magnesium (as MgO) in a series of typical flours as reported by Dempwolf was computed by the present author, and these lend emphasis to this general conclusion.

Flour grade	Total ash (%)	CaO/MgO in the ash
0	0.38	1.15
2	0.45	0.96
4	0.59	0.66
6	0.76	0.61
8	1.55	0.37

Wheat analyses in the number of 1756 were consulted by Word and Wakeham,⁴ and the following ranges in calcium, iron, phosphorus and sulfur were encountered:

³ *Ann. Chem. Pharm.*, 149, 343 (1869).

⁴ *Univ. Colo. Studies*, 25, 181 (1938).

	Minimum	Maximum
Calcium (%)	0.028	0.296
Iron (%)	0.001	0.0052
Phosphorus	0.408	0.425
Sulfur	0.009	0.224

Depending upon the sources from which these data were drawn, the significance of mere ranges is open to doubt. Some of the older analyses may be questioned as to accuracy, and "freak" samples which are not especially important in themselves may serve to establish the extremes thus recorded.

Bailey and Hutchinson⁵ surveyed the iron, copper, calcium, and magnesium content of 44 typical hard spring wheats, chiefly of the Marquis and Ceres varieties, grown in the northwestern states—chiefly in the crop year 1933. The maximum, minimum, average, and coefficient of variation of each mineral and of the total ash are recorded in Table 70

Table 70. Copper, Iron, Calcium, Magnesium and Total Ash Content of Northwestern Grown Hard Spring Wheats, Crop of 1933, on the Dry Matter Basis, as Reported by Bailey and Hutchinson.⁵

	Copper (%)	Iron (%)	Calcium (%)	Magnesium (%)	Total ash (%)
Average	0.00099	0.0055	0.0525	0.2285	1.965
Maximum	0.00115	0.0111	0.0710	0.2900	2.525
Minimum	0.00075	0.0041	0.0400	0.1510	1.518
Coefficient of variation	15.5	20.0	29.5	14.4	15.2

on a dry matter basis. Each entry of percentage should be multiplied by the factor 0.865 to correct to a 13.5 per cent moisture basis. It appears that calcium was the most variable of these four minerals, followed by iron, copper and magnesium in the order named.

In addition to the data here recorded, the correlations of the percentages of the four individual minerals with the percentage of total ash were computed. Using the symbol A for ash in each instance, these coefficients of correlation were as follows:

$$\begin{aligned} r_{A.Cu} &= 0.12 \\ r_{A.Fe} &= 0.55 \\ r_{A.Ca} &= 0.29 \\ r_{A.Mg} &= 0.75 \end{aligned}$$

It will be noted that the element which exhibited the least variability, viz. magnesium, was most highly correlated with the percentage of total ash; in fact, it is the only element which could be predicated from the ash content with even a fair degree of accuracy.

The ratios between iron and copper and between magnesium and

⁵ Data recorded in the Division of Agricultural Biochemistry, University of Minnesota.

calcium were also computed in each instance. The Fe/Cu ratio averaged 5.64 with a coefficient of variation of 24.1, while the Mg/Ca ratio averaged 4.59, with a coefficient of variation of 22.4. The coefficient of correlation r Fe.Cu was only 0.29, and r Mg.Ca=0.02, which discloses that none of these elements can be estimated from the percentage of the other member of the pair.

The values for iron recorded in these and similar studies have been criticized on the score that the analyses were not conducted in an "iron-free" laboratory. Accordingly the results may be somewhat too high, as suggested by the average value accepted by the Council on Foods of the American Medical Association,⁶ which is 0.0039 per cent (=3.9 mg/100 g) for whole wheat.

In the culture of wheat the ash content was found by Kniaginichev⁷ to be increased by additional soil moisture or by increased solar radiation. Ash content was lower in arid regions. Vitreous winter wheat kernels contained more ash than starchy or yellow-berry kernels, as was also true of flour milled from the two types of kernels. Winter wheat flour was lower in ash content than spring wheat flours of the same degree of extraction, and even more so on comparing it with *durum* wheat flour. Ash content of flours tended to vary closely with that of the wheats from which they were milled when comparisons were restricted to flours milled in the same manner.

It is possible that the low ash content of wheats grown in New South Wales, and Victoria, Australia, namely 1.45 per cent, as reported by Dadswell,⁸ may be accounted for on the basis of Kniaginichev's hypothesis.

Severe weathering, involving exposure of unthreshed stems of wheat in the shock, or scattered in trays, throughout an entire winter, did not result in a significant change in total ash content of the wheat, or of flour produced therefrom, in the experiments conducted at Bozeman, Montana by Whitcomb and Johnson.⁹

Ash analyses of nearly four thousand samples of Rumanian wheats of the six crops of 1900 to 1905 inclusive, as reported by Zaharia,¹⁰ were subjected to statistical analysis by Berczeller and Wastl.¹¹ It appeared that the ash content was less variable, relatively, than the percentage of protein, but more variable than fat or cellulose content. The coefficient of variation of the combined data of the six crops was 10.4 per cent, as

⁶ *J. Am. Med. Assn.*, 116, 2849 (1941).

⁷ Report No. 5 of the Inst. Appl. Bot. of the Lenin Acad. of Agr. (U.S.S.R.) 1934. With English summary.

⁸ *Australian J. Exptl. Biol. Med. Sci.*, 13, 33 (1935).

⁹ *Cereal Chem.*, 7, 162 (1930).

¹⁰ "Le blé Roumain," Bucharest, 581 pp. 1910.

¹¹ *Biochem. Z.*, 177, 168 (1926).

computed later by Immer.¹² The modal class, when the data were grouped in classes ranging through 0.1 per cent of ash each, was the one averaging 2.05 per cent, and the mean was 2.02 per cent. All data were recorded on the water-free basis.

In the instance of 1,177 samples of No. 1 northern Canadian spring wheat of the crop of 1933, Geddes¹³ found the average ash content to be 1.55 per cent. The standard deviation was 0.16, and the coefficient of variability accordingly became 10.1 per cent. Over 60 per cent of the samples contained between 1.40 and 1.65 per cent of ash.

A high correlation, $r=0.81 \pm 0.02$, was observed by Sherwood and Bailey¹⁴ between the ash content of 148 spring wheats, and of the straight-grade flour milled therefrom. When the wheat samples were sorted into groups, each with a unit range of ash content, and the average ash of these groups was plotted against the average ash of straight-grade flours milled from these same wheats, the result was a straight line. For the mill in question, as shown by Naszalyi's¹⁵ computation, the relation between ash in wheat (A_w) and ash in straight grade flour (A_s) was expressed by the formula:

$$\frac{A_s}{A_w^{\frac{2}{3}}} = 0.355$$

This affords a basis for the prediction of ash content of flour from ash content of wheat, and is a useful correction for estimating degree of extraction from the ash content of flour, when the ash content is known and varies appreciably from the average of the class of wheat from which the flour was milled.

Table 71. Mineral Content of Egyptian and Other Wheats,
Data of Schrumpf-Pierron.¹⁶

Variety	K ₂ O (%)	CaO (%)	MgO (%)	P ₂ O ₅ (%)
1. "Hindi," Egyptian	0.409-0.474	0.069-0.071	0.222-0.249	0.656-0.807
2. "Baladi," "	0.482-0.508	0.056-0.061	0.196-0.222	0.804-0.817
3. Manitoba	0.412	0.063	0.210	0.676
4. Institute Pasteur de Lille, average	0.450	0.044	0.176	0.690
5. Hybrid 23, France	0.472	0.098	0.150	0.659

Egyptian wheats studied by Schrumpf-Pierron¹⁶ were found to contain the percentages of certain minerals shown in Table 71. Schrumpf-Pierron attached considerable significance to the ration $MgO/K_2O + CaO$

¹² Private Communication, July, 1941.

¹³ Dom. Grain Research Lab. (Canada), 9th Annual Rept. p. 74, 1935.

¹⁴ *Cereal Chem.*, 5, 437 (1928).

¹⁵ *Z. ges. Mühlenw.*, 7, 99 (1930).

¹⁶ *Bull. inst. Egypt*, 14, 147 (1932).

and observed that this ratio reached its highest value in the superior Egyptian variety "Hindi", and was lower in the inferior variety, "Baladi". From the alimentary standpoint it is desirable, in his opinion, for this ratio to exceed unity. Among the five samples included in this table the ratio was as follows: (1) 0.91—1.03; (2) 0.79—0.94; (3) 0.95; (4) 0.78; (5) 0.52.

In an extension of these studies of Egyptian wheat Schrumpf-Pieron¹⁷ included twelve samples of new wheat varieties in his analyses. The K_2O content ranged from 0.305 to 0.490 per cent, averaging 0.411 per cent; the CaO content from 0.057 to 0.109 per cent, averaging 0.084 per cent; the MgO content from 0.079 to 0.310 per cent, averaging 0.173 per cent; the P_2O_5 content from 0.466 to 0.728 per cent, averaging 0.614 per cent; and the $MgO/K_2O + CaO$ ratio from 0.35 to 1.17, averaging 0.745.

The concentration of several of the trace elements, as well as the more prominent mineral constituents in wheat and its typical mill products, was computed by Sullivan and Near¹⁸ from their analyses of the ash of these materials. Some of their data are recorded in Table 72.

Table 72. Mineral Content of Wheat and Mill Products Calculated as the Elements from Analysis of the Ash by Sullivan and Near.¹⁸

Element	In parts per million of product, on dry basis						
	Wheat	Patent flour	Clear flour	Low-grade flour	Total mill-run middlings	Bran	Germ
Total ash	20,500	4,820	8,040	14,620	47,620	67,480	50,410
Magnesium	1,898	308	624	1,327	4,546	7,166	3,801
Calcium	452	180	227	376	1,115	1,158	692
Phosphorus	4,440	1,162	1,910	3,511	10,446	15,208	12,533
Potassium	2,370	552	875	1,553	5,633	7,098	5,542
Zinc	100	40	48	129	319	562	420
Iron	31	8	11	22	71	95	68
Manganese	24	2	5	12	48	112	67
Copper	6	2	2	4	13	14	9
Aluminum	3	0.6	2	7	8	27	25

They emphasize that the so-called catalytic elements, Fe, Mn, Zn, Cu, etc., are most prominent in those parts of the wheat kernel that are richest in enzymes. In another portion of their paper appear data which establish that the percentage of magnesium (as MgO) in the ash increased in progressing from the more highly refined products through the lower grade and coarse streams, while at the same time the calcium content (as CaO) decreased. They also concluded that the phosphorus is present in the ash chiefly as pyrophosphate, together with some metaphosphates. The proportion of the latter increased with the per-

¹⁷ *Compt. rend. soc. biol.*, 111, 847 (1932).

¹⁸ *Ind. Eng. Chem.*, 19, 498 (1927).

centage of phosphate, and accordingly reached its highest proportion in the ash of the more refined or patent flour (55.2 per cent P_2O_5 in the ash) where it represented about 1 part to 4 parts of pyrophosphates. Such ashes had the lowest fusion temperature, and are most difficult to recover free from carbon.

In a later communication Howe and Sullivan¹⁹ reported on additional studies of the potassium and sodium content of wheat and its mill products. The concentration of the former was about twice that previously reported by Sullivan and Near.¹⁸ The percentages in these materials, calculated to a dry basis, were as follows:

	Potassium (%)	Sodium (%)
Wheat	0.520	0.003
Patent flour	0.109	0.001
Clear grade flour	0.174	0.002
Bran	1.539	0.005
Germ	1.151	0.004

The mineral content of grains received the special attention of Greaves and his colleagues at the Utah Agricultural Experiment Station. In one of the early publications in the series, Greaves and Hirst²⁰ reported 0.053 to 0.109 per cent of calcium in different varieties of wheat grown on the Nephi plots, although a wider range of 0.028 to 0.296 per cent was encountered when wheats from different localities in Utah were compared. There was a tendency toward an increase in the calcium content of wheat as the application of irrigation water was increased, and the same was true, although to a less marked degree, in the instance of

Table 73. Minimum, Maximum and Average Percentage of Certain Constituents of Wheats Grown at the Utah Agricultural Experiment Station, as Reported by Greaves and Hirst.²¹

	Minimum (%)	Maximum (%)	Average (%)
Total ash	1.350	2.940	1.850
Calcium	0.028	0.296	0.090
Magnesium	0.131	0.276	0.184
Potassium	0.307	0.960	0.476
Phosphorus	0.150	0.458	0.331
Sulfur	0.120	0.287	0.204
Iron	0.001	0.032	0.005

the magnesium content. A variation of 212 per cent in the magnesium content of wheats from different localities in Utah was observed, the range being from 0.135 to 0.278 per cent, average 0.184 per cent. Potassium content ranged from 0.409 to 0.971 per cent, average 0.476 per cent; total phosphorus from 0.205 to 0.455 per cent; sulfur from 0.120 to

¹⁹ *Cereal Chem.*, 13, 61 (1936).

²⁰ *Utah Agr. Exp. Sta. Bull.* 210 (1929).

0.237 per cent, average 0.204 per cent; iron from 0.0037 to 0.0159 per cent, average 0.00697 per cent.

Greaves and Hirst²¹ assembled the analyses of 1755 wheat samples grown at the Utah Agricultural Experiment Station; the minimum, maximum, and average of their content of total ash, calcium, magnesium, potassium, phosphorus, sulfur and iron are recorded in Table 73.

The application of irrigation water increased the iron, chlorine and sulfur content of wheat grown on the Greenville (Utah) Experiment Farm, according to the findings of Greaves and Nelson.²² Some of their data are recorded in Table 74.

Table 74. Certain Constituents of Wheat Grown with Varying Amounts of Irrigation Water and Analyzed by Greaves and Nelson.²²

Treatment	— Milligrams per 100 grams —		
	Iron	Chlorine	Sulfur
No irrigation water	19	33	144
15 inches irrigation water	25	75	197
35 inches irrigation water	32	89	213

In moisture-free mill products McHargue²³ found the following metals, recorded as parts per million:

	Wheat bran	Wheat germ	Patent flour
Copper	16.0	46.0	trace
Iron	210.0	270.0	24.0
Manganese	125.0	150.0	10.0
Zinc	75.0	160.0	trace

An extensive study of the minerals in English foods has been made by McCance and Widdowson, and a summary of their findings was recently published.²⁴ The average in milligrams per ounce of certain mineral elements in white flour and wholemeal flour as reported by them was as follows:

	White flour	Wholemeal flour
Sodium	8.4	45.4
Potassium	41	92
Calcium	5.2	8.2
Magnesium	6.7	28.7
Iron	0.26	0.84
Copper	0.02	0.11
Phosphorus	28.8	69.7
Sulfur	30.9	35.0
Chlorine	19.2	50.3

The manganese, iron, and copper content of serving portions of cooked foods was computed by Hodges and Peterson.²⁵ In slices weighing 25 g

²¹ *J. Nutrition*, 1, 293 (1929).

²² *Soil Sci.*, 19, 825 (1925).

²³ *J. Agr. Research*, 30, 193 (1925).

²⁴ "The Chemical Composition of Food," 150 pp. New York, 1940.

²⁵ *J. Am. Dietet. Assoc.*, 7, 6 (1931).

each, the following quantities of these minerals were encountered in the types of bread listed:

	Milligrams of		
	Mn	Cu	Fe
Graham bread	0.79	0.08	0.63
White bread	0.08	0.09	0.23
Rye bread	0.32	0.07	0.48

In a tablespoon of flour, there were the following amounts:

Tablespoonful of	Weight (g)	Milligrams of		
		Mn	Cu	Fe
Graham flour	9	0.39	0.04	0.33
Patent flour	7	0.03	0.01	0.06

In one-half cup each of cooked whole wheat (25 g) and of bran (40 g) there were 0.91 and 3.41 mg of iron respectively, and 0.86, and 3.64 mg of manganese.

The range in concentration of certain elements in the ash of winter wheat, as determined by Klemm, was reported by Schmorl.²⁶ The data are as follows, in terms of percentage of the ash:

	Maximum	Minimum
Potash	31.7	25.2
Lime	4.6	4.0
Magnesia	11.8	8.9
Iron	2.4	0.4
Phosphoric acid	52.6	48.0
Silicic acid	5.5	1.5
Sulfuric acid	2.0	1.2
Chlorine	1.2	0.4
Soda	2.2	0.8

Although the percentage of iron and phosphorus appeared to vary fairly regularly with the ash content, in the analyses of flour mill streams made by Harding and Dysterheft,²⁷ the content of calcium and magnesium did not exhibit so definite a correlation. Thus the percentage of calcium in the ash of these streams varied from 1.81 per cent in the second break flour to 3.95 per cent in the first middlings flour. This is a range in the ratio of Ca/total ash of more than 100 per cent. The range in the Mg/total ash ratio was not quite as wide proportionately as just reported for calcium, the extremes being 5.31 per cent in the first middlings, and 8.62 per cent in the fourth break. The computations of these authors indicate that, in general, the Ca/Mg ratio reaches a maximum in the most highly refined flour streams such as the first and third middlings, where it is 0.74 and 0.68 respectively, and is at a much lower level in the break flour streams, the minimum of their series being the fourth break where this ratio reached 0.22.

²⁶ *Z. ges. Getreidew.*, 20, 300 (1933).

²⁷ *Cereal Chem.*, 4, 47 (1927).

The phosphorus/protein ratio was at a higher level in the lower-grade flour streams, and in the clear flour, than in the more highly refined middlings streams and the patent flour. The basic data involved in these calculations are recorded in Table 75.

Table 75. Percentage of Ash, Iron, Calcium, Magnesium, and Phosphorus in Certain Flour Mill Streams, as Reported by Harding and Dysterheft.²⁷

Wheat	Ash	Percentages in flour streams on oven-dried basis			
		Fe	Ca	Mg	P
First break	0.683	0.00079	0.0184	0.0545	0.168
Second break	.668	.00069	.0121	.0493	.158
Third break	.841	.00099	.0177	.0688	.198
Fourth break	1.344	.00151	.0250	.1158	.321
First middlings	0.418	.00036	.0165	.0222	.091
Second middlings	.396	.00037	.0100	.0219	.097
Third middlings	.416	.00041	.0164	.0243	.109
Fourth middlings	.448	.00047	.0099	.0276	.115
Straight flour	.512	.00050	.0114	.0318	.132
Clear flour	.810	.00088	.0275	.0613	.197
Wheat	1.761	.00184	.0317	.1543	.384
Bran	4.868	.00550	.0965	.4505	1.134

An analysis of wheat germ by Zunini²⁸ disclosed the following percentages of constituents: ether extract 8.20 (dry basis); ash 4.25; nuclein phosphorus 0.368 per cent; phytin phosphorus 0.34; lecithin phosphorus 0.10; potassium 1.09; sulfur 0.24; magnesium 0.16; calcium 0.043; sodium 0.01; and silicon 0.009. Zinc, manganese, iron, nickel, aluminum, copper, cobalt, and boron were also detected.

Silicic acid content of entire wheat was found by Strohecker, Vaubel and Breitwieser²⁹ to be low, namely, 0.13 per cent, while the percentage in wheat starch was considerably higher, namely 0.22 per cent. Even more significant is the percentage of SiO₂ in the ash of wheat, and wheat starch, which is 0.66, and 9.09 per cent, respectively. These investigators conjecture that the silicic acid is chiefly contained in the starch of wheat, and quite evidently in the amylopectin fraction.

From the compilation of data published by Beeson³⁰ the means of mineral analyses of wheat have been selected and are recorded in Table 76.

The range in percentage of various elements in wheat and in white flour, as recorded in the literature, was reported by Booth, Carter, Jones, and Moran.³¹ These data in Table 76 indicate a considerable variation in the concentration of these elements, which are attributed by these reviewers to the effect of soil, conditions of growth, water supply, time

²⁸ *Chim. ind. agr. biol.*, 11, 339 (1935).

²⁹ *Z. ges. Getreidew.*, 22, 131 (1935).

³⁰ *U. S. Dept. Agr., Misc. Pub.* 369 (1941).

³¹ *Chem. and Ind.*, 60, 905 (1941).

Table 76. Mineral Composition of Wheat and of Flour, as Compiled by Beeson⁸⁰ and by Booth, Carter, Jones and Moran.⁸¹

Element	Data of Beeson ⁸⁰			Compilation of Booth <i>et al.</i> ⁸¹	
	Citation No.	No. of samples	Mean, whole wheat (mg/100 g)	Range, whole wheat, dry basis (mg/100 g)	Range, white flour, dry basis (mg/100 g)
Potassium		264	480±60	340-580	100-170
Sodium		100	70*	3-9	2-4
Calcium		290	50±21	27-250	13-27
Magnesium		267	170±40	115-236	22-30
Phosphorus		310	400±70	173-428	102-130
Sulfur		138	180±50	102-247	100-165
Arsenic	32		0.015	0.01	0.001
	33		0.030		
Barium	34		0.8		
Boron (above ground portion)	35		0.3	0.07-0.50	0.05
Chlorine		80	90	50-170	50-67
Iodine		26	0.0067	Trace	Trace
Bromine				0.2	0.1
Cobalt	36		0.0012	0.01	0.008
Copper		108	0.9	0.4-3.0	0.07-0.20
Iron		131	6.8	3-5	0.8-1.1
Manganese		109	4.9	2.4-5.0	0.3-1.0
Titanium	37		0.09	0.08	
Zinc		27	6.3	10	4
Silicon				6	0.5
Nickel				3.5	0.13
Lithium					0.17
Tin					0.13

*This figure appears to be very high, even on a dry matter basis, when compared with other data.

of sowing, as well as varietal differences. They also analyzed three samples of wheat for their calcium and iron content, and three mill products for their calcium content with the following results:

	Total calcium on 15 % moisture basis (mg/100 g)	Total iron, on 15 % moisture basis (mg/100 g)
Manitoba wheat	32.4	4.60
Plate wheat	41.7	5.14
English wheat	43.7	4.01
Mixed wheat	38.4	
Bran	90.4	
Germ	46.7	
White flour	15.9	

Ash elements were determined by spectrographic analysis in a collection of flours examined by Morris, Bode, Hartsing, and Heizer.³⁸ Their results, as shown in Table 77, indicate that phosphorus and potassium

⁸² Mitt. Gebieten Lebensm. Untersuch. Hyg., 20, 338 (1929).

⁸³ U. S. Dept. Agr. Tech. Bull. 732 (1940).

⁸⁴ Soil Sci. Soc. Proc., 3, 87 (1938).

⁸⁵ Ann. Southern Agr. Workers Proc. 37, 257, (1937).

⁸⁶ Bull. soc. chim. (4), 47, 326 (1930).

⁸⁷ Ibid. 45, 1044 (1929).

⁸⁸ Paper entitled "A study of the effect of ash constituents on flour viscosity," presented before the 1941 convention of the Amer. Assn. of Cereal Chemists, Omaha, Nebraska.

content of these flours increased fairly regularly with their ash content. The sodium content, as reported here for the short extraction flours, is considerably higher than has usually been given for flours, being in about the same range as the magnesium content. It did not increase as much as did the magnesium when the ash content was increased by the addi-

Table 77. Percentage of Ash and Certain Ash Elements of Flours of Varying Extraction (basis of 13.5 per cent moisture), as Reported by Morris, Bode, Hartsing and Heizer.³⁸

Flour traction (%)	Ash (%)	P (%)	Mg (%)	K (%)	Ca (%)	Na (%)	Mn (%)
62.3	0.35	0.094	0.026	0.097	0.004	0.020	0.00031
72.0	0.40	0.116	0.044	0.098	0.006	0.024	0.00056
75.0 ¹	0.45	0.138	0.056	0.120	0.005	0.028	0.00066
75.0 ²	0.50	0.148	0.069	0.133	0.007	0.035	0.00090
*	0.30	0.088	0.032	0.095	0.005	0.026	0.00042

¹ Low-grade flour added to bring ash content from 0.43 to 0.45.

² Low-grade flour added to bring ash content from 0.44 to 0.50.

* Unbleached commercial cake flour.

tion of low-grade flour. No large change in calcium was registered by raising the ash content, but the quantity is small in all instances. Manganese trebled while the ash content was being increased only half that much proportionately.

PHOSPHORUS AND PHOSPHORUS COMPOUNDS

The correlation between the phosphorus content of flour mill streams and other constituents, including acidity, amino compounds, and ash, was traced by Swanson.³⁹ He proposed that at least a part of the water-soluble acid-reacting substances in a flour extract was mono-potassium phosphate, which would account in large measure for the relationship between soluble phosphorus and acidity.

The phosphorus content of the ash of several hundred flours analyzed by Johnson and Scott,⁴⁰ calculated as P_2O_5 , was observed to range from at least 47.95 to 54.50 per cent. Moreover, the high-phosphorus ash exhibited a tendency to fuse more readily under the conditions of incineration they employed, *i.e.*, a 16-hour period at 585° C. At the same time the potassium content was lowest in the high-phosphorus ash which fused so readily. They discuss the possible forms of phosphates which may occur in the ash, and conclude that most of the phosphorus is present in the pyrophosphate form, supporting the view advanced earlier by Sullivan and Near.¹⁸

The phosphorus (as P_2O_5) content of the ash was observed by Eble and Bretschneider⁴¹ to increase somewhat in progressing from the more

³⁹ *J. Ind. Eng. Chem.*, 4, 274 (1912).

⁴⁰ *Cereal Chem.*, 5, 56 (1928).

⁴¹ *Z. untersuch. Lebensmittel.*, 66, 314 (1933).

highly refined to the lower-grade rye flours. In the refined or low-ash flours it averaged 34.3 per cent of the ash, and ranged upward to 51.5 per cent in the lower-grade or high-ash flours.

The Council on Foods of the American Medical Association⁴² accepted an average value of 1700 mg of phosphorus per pound (=374 mg/100 g) for whole wheat and 460 mg/lb (=101 mg/100 g) for white flour.

An increase in organic phosphorus in progressing from the high-grade flour to the bran was observed by Masoni.⁴³ This paralleled the increase in ash and fat content. As shown by the data in Table 78, the major change, both in proportion and in absolute quantities, was in phytin content. Lecithin content varied slightly among the mill products; nuclein phosphorus increased somewhat in progressing from flour to bran, but not to the degree that was observed with phytin.

Table 78. Phosphorus Distribution in Flour Mill Products from a Roller Mill, as Reported by Masoni.⁴³

Material and percentage	Moisture (%)	Fat (%)	Ash (%)	Phosphorus as P_2O_5 in			
				Lecithin (%)	Phytin (%)	Nuclein (%)	Total (%)
Cleaned wheat	15.05	1.66	1.76	0.032	0.609	0.130	0.860
Flour, 70 %	15.66	0.68	0.48	0.026	0.110	0.100	0.264
Low-grade flour, 5 %	14.20	1.40	1.32	0.026	0.231	0.150	0.480
Light shorts, 3 %	14.08	3.30	3.64	0.033	0.974	0.204	1.971
Dark shorts, 4 %	14.94	3.68	4.30	0.040	1.254	0.206	2.304
Bran, 18 %	14.50	3.50	5.90	0.038	2.457	0.283	2.994

A perfect correlation between the total ash and the phosphorus content of flour and mill products was not encountered by Proskuryakov and Temerin,⁴⁴ although the agreement between these two variables was fair.

The phosphorus content of wheat receiving irrigation tended to increase in the experiments of Greaves and Hirst⁴⁵ until 35 inches of water was reached, and then receded on further increasing the irrigation. In wheat an average of 6.3 per cent of the phosphorus was found to be in inorganic combination, when determined by the Forbes method.⁴⁶

The total phosphorus (as P_2O_5) content of the developing wheat grain was observed by Rousseaux and Sirot⁴⁷ to remain practically constant between June 23 and July 26, when it was harvested. The percentage of the total P_2O_5 which was "soluble" decreased sharply, however, from 77 per cent on the first date to 36 per cent at ripeness.

After criticizing certain of the methods employed in the determination

⁴² *J. Am. Med. Assn.*, 116, 2849 (1941).

⁴³ *Staz. sper. agr. ital.*, 48, 385 (1915). Abst. in Winton and Winton, "The Structure and Composition of Foods," Vol. 1, p. 231, 1932. Original not seen.

⁴⁴ *Sci. Inst. Cereal Research (U. S. S. R.)* 12, 32 (1933).

⁴⁵ *Cereal Chem.*, 6, 115 (1929).

⁴⁶ *Ohio Agr. Exp. Sta. Bull.* 215, p. 459 (1910).

⁴⁷ *Compt. rend.*, 171, 578 (1920).

of the phosphorus content of flour, including Neumann's, and Spaeth's methods, Kocsis and Hegudus⁴⁸ applied the Simmich mass analysis method to flour and obtained higher results than with the two other methods mentioned. The differences were not large, and particularly in comparison with Spaeth's method, but Kocsis and Hegudus felt there were other advantages in favor of the method which they employed.

In all the cereals studied by Posternak⁴⁹ he found 70-90 per cent of the phosphorus to be contained in a phospho-organic acid or its salts. Lecithin represented only 7 per cent or less of the phosphorus present. These salts were found by him⁵⁰ to be compounds of magnesium and calcium, with a little iron and manganese. The free acid was soluble in all proportions in distilled water, somewhat soluble in absolute alcohol, insoluble in ether, benzene, chloroform, and glacial acetic acid. Its empirical formula corresponded to $C_2H_8P_2O_9$.

Later Posternak⁵¹ indicated the recovery of inosite and phosphoric acid from his phospho-organic acid and proposed two possible formulas which were described as anhydro-oxymethylenediphosphoric acid. He also speculated concerning its possible role in photosynthesis.

After noting that 86.5 per cent of the phosphorus of wheat bran appeared in a water extract, and 77.8 per cent in an extract made with dilute HCl, Patten and Hart,^{52, 53} attempted to isolate the principal phosphorus compound. Alcohol was added to the extract made with 0.2 per cent HCl, and the precipitate was dissolved in dilute HCl and reprecipitated with alcohol. This process was repeated, and the resulting preparation was subjected to analysis. Inosite and phosphoric acid appeared upon treatment with concentrated mineral acids. This, together with its elementary composition, caused them to classify it with Posternak's⁵⁴ "anhydro-oxymethylene-diphosphoric acid." In the original bran it was presumed to exist as the magnesium-calcium-potassium salt. Since practically all of the phosphorus in the dilute HCl extract appeared in this form, it would seem that Patten and Hart assumed that three-fourths of the phosphorus of bran is included in this substance.

Phosphorus compounds soluble in 0.2 per cent HCl were also studied by Rather.⁵⁵ Copper acetate was added to the acid extract, and the precipitate was purified as a barium compound. Silver salts were also prepared. The composition of the acid corresponded to $C_{12}H_{41}P_9O_{42}$ which is not in accord with the results of Patten and Hart.⁵³ Rather

⁴⁸ *Z. Untersuch. Lebensm.*, 70, 474 (1935).

⁴⁹ *Compt. rend.*, 137, 202 (1903).

⁵⁰ *Ibid.*, 137, 337 (1903).

⁵¹ *Ibid.*, 137, 439 (1903).

⁵² *Am. Chem. J.*, 31, 564 (1904).

⁵³ New York (Geneva) Agr. Expt. Sta. Bull. 250 (1904).

⁵⁴ *Rev. gen. botan.*, 12, 5, 65 (1900).

⁵⁵ *J. Am. Chem. Soc.*, 35, 890 (1913).

felt that this formula represented the empirical formula of the phytic acid of bran.

The phosphorus in the lipoids represented a larger percentage of the total phosphorus in the refined or patent flour than in the lower-grade mill products or the wheat from which they were milled, in the analyses made by Sullivan and Near.⁵⁶ The lipid P/total P ratio for these several materials was: wheat 0.041; patent flour 0.128; clear flour 0.079; low-grade flour 0.056; bran 0.18; germ 0.040.

The percentages of several inorganic constituents of wheat grown in 1930 at Good Easter, Essex, England, as well as the nitrogen content, were reported by Knowles and Watkin.⁵⁷ The following data are recorded as percentages of the dry matter of the grain:

Nitrogen	1.574
Phosphoric acid	0.936
Lime	0.085
Potash	0.504
Chlorine	0.050

These same investigators also traced the changes in composition of the wheat plant, and of its grain and straw, with its progressive development from April 30 until harvest on August 6.

In a later communication Knowles and Watkin⁵⁸ reported the distribution of phosphorus in lipin, phytin and inorganic combinations in the ears of the wheat plant from six samplings beginning on June 18, 1930 and extending to the harvest on August 6. From their data, as recorded in Table 79 it is evident that the inorganic phosphorus which predominated in the earlier samplings, was transformed largely into phytin combinations, whereas the lipin phosphorus, though it actually increased in quantity, reached a lower percentage of the total phosphorus as maturation advanced because of dilution of the mixture with phytin and inorganic phosphorus; of the organic phosphorus compounds phytin contained the bulk of this element.

The total and lipid phosphorus content of wheat and its mill products, and the phytosterol content were reported by Leulier and Chevat,⁵⁹ and the results of their analyses are recorded in Table 80.

A larger proportion of the total phosphorus was in lipid combination in the flour than in the bran and shorts, with the entire wheat in an intermediate position. The phytosterol content more or less followed the degree of refinement of the material, being lowest in the flour, intermediate in the entire wheat, and greatest in the coarse feeds.

⁵⁶ *Cereal Chem.*, 5, 163 (1928).

⁵⁷ *J. Agr. Sci.*, 21, 612 (1931).

⁵⁸ *Ibid.*, 22, 758 (1932).

⁵⁹ *J. pharm. chim.*, 14, 214 (1931).

Table 79. Weight and Percentage of Phosphorus in Wheat Ears, as Sampled during Progressive Development from June 18 until August 6, 1930, as Reported by Knowles and Watkin.⁵⁸

Sampling date (1930)	Weight in grams per 3200 tillers (as P_2O_5)			
	Total	Lipin	Phytin	Inorganic
June 18	10.94	0.97	0.91	9.06
July 2	23.01	1.00	9.16	12.84
July 16	44.51	1.15	22.02	21.34
July 23	50.86	1.17	24.78	24.91
July 30	53.61	1.33	28.45	23.83
Aug. 6	54.07	1.44	26.68	25.95

	Percentage of dry matter of ears (as P_2O_5)			
	Total	Lipin	Phytin	Inorganic
June 18	0.941	0.084	0.079	0.778
July 2	0.952	0.051	0.379	0.522
July 16	0.912	0.024	0.451	0.437
July 23	0.800	0.020	0.390	0.390
July 30	0.807	0.020	0.418	0.369
Aug. 6	0.821	0.024	0.405	0.392

	Percentage of total phosphorus in the ears		
	Lipin	Phytin	Inorganic
June 18	8.9	8.4	82.7
July 2	5.4	39.8	54.8
July 16	2.6	49.4	48.0
July 23	2.5	48.7	48.8
July 30	2.5	51.8	45.7
Aug. 6	2.9	49.3	47.8

In a collection of five wheat samples, at least four of which were hard red winter wheat, Guerrant⁶⁰ found from 0.0272 to 0.0332 per cent of lipid phosphorus, the average being 0.0306 per cent. In these same samples the average total phosphorus content was 0.289 per cent, ranging from 0.262 to 0.314 per cent.

Table 80. Total Phosphorus, Lipid Phosphorus and Phytosterol Content of Wheat and Mill Products, as Reported by Leulier and Chevat.⁵⁹

Material	Percentage of total wheat	Moisture (%)	Phosphorus		Phytosterol (%)
			Total (%)	Lipid (%)	
Entire wheat	100	11.4	0.40	0.014	0.060
Flour	75	11.9	0.13	0.010	0.035
Coarse bran	11	10.7	1.41	0.020	0.131
Fine bran	3	9.8	1.19	0.019	0.082
Shorts	11	10.4	0.90	0.023	0.129

After developing an improved method for the determination of inorganic phosphorus in plant materials, Collison⁶¹ applied it to wheat

⁶⁰ *J. Agr. Research*, 35, 100 (1927).

⁶¹ *J. Biol. Chem.*, 12, 65 (1912).

grains, bran, and germ and found 0.012, 0.034, and 0.040 per cent, respectively.

In the process of recovering wet crude gluten by washing it from the dough, 45.3 per cent of the phosphorus appeared in the starch, 24.5 per cent in the gluten, and 30.2 per cent in the wash water, in the experiments of Nottin and Dartois.⁶² A flour containing 0.513 per cent total ash, on more detailed analysis was found to contain 0.024 per cent of silica, 0.042 of iron and aluminum oxides, 0.037 of lime, 0.034 of magnesium, 0.219 of phosphoric acid, 0.138 of potassium, and 0.027 of sodium.

A study of the methods for determining phytin phosphorus in plant products was made by Rather.⁶³ He found that 1.2 per cent HCl exerted a better solvent power for phytin phosphorus than did 0.2 per cent HCl, and at the same time prevented enzymic hydrolysis of the phytin. The ferric chloride titration method for the determination of phytin phosphorus in pharmaceutical products was applicable to extracts of plant products. When thus determined, the phytin phosphorus content of a sample of wheat bran was 1.24 per cent, which was 88 per cent of the total phosphorus; there was 0.61 per cent of phytin phosphorus in wheat shorts, which was 0.66 per cent of the total phosphorus, and 84 per cent of the acid-soluble phosphorus. Agreement between triplicated analyses was good, the range being within 0.02 per cent in the instance of bran, and 0.06 per cent in the shorts.

Phytin was extracted from wheat bran by Boutwell⁶⁴ with 2 per cent HCl at room temperature. The extract was heated to coagulate the proteins, and ammonia added to faint alkalinity with continued boiling. The resulting precipitate was filtered off while hot, and was then extracted with 8 per cent acetic acid. When this was heated to boiling, a large quantity of phytin separated out as a fine white precipitate, which redissolved on cooling. This solution was filtered, diluted with an equal volume of water, heated to boiling, and again made alkaline with ammonia. The copious white precipitate was recovered by filtering while hot, and was washed with boiling water. This precipitate was extracted with the least possible quantity of 0.8 per cent acetic acid, and on heating the acid extract to boiling, the phytin separated out as a heavy, white, pulverant precipitate. This was recovered by filtering while hot, washed with hot water, alcohol, and ether, and dried at room temperature. A yield of about 25 g was obtained from the original charge of somewhat more than 1 kg. It was insoluble in hot and cold water, readily soluble in dilute mineral acids and in cold, dilute acetic acid, but was repre-

⁶² *Compt. rend. acad. agr. France*, 20, 989 (1934).

⁶³ *Ark. Agr. Exp. Sta. Bull.* 135 (1917).

⁶⁴ *J. Am. Chem. Soc.*, 39, 491 (1917).

precipitated on heating its solution in the latter to boiling. Its barium salts were recovered from a 2 per cent HCl solution, and on decomposing them with H_2SO_4 from an HCl solution, the copper salts could be recovered by adding copper acetate.

The barium salts, as crystallized from dilute HCl on boiling, agreed with a mixed salt consisting of two molecules of tri-barium inosite hexaphosphate and one molecule of mono-barium inosite tetraphosphate.

In a series of papers on phytin and related substances, Anderson⁶⁵ begins with a description of the salts of phytic acid, or the phytates. This phase of the subject was further expanded in the second paper.⁶⁶ In the third paper of the series Anderson⁶⁷ described the recovery of a substance from wheat bran by extracting it with 0.2 per cent HCl, and precipitation from the filtered extract with alcohol. After repeated reprecipitation he obtained a material of relatively uniform composition, averaging C, 21.0 per cent; H, 3.5 per cent; P, 14.0 per cent. It contained calcium, magnesium, potassium and sodium in varying amounts, traces of iron, and always contained some nitrogen, varying around 0.4 to 2.1 per cent. It reduced Fehling's solution, yielded furfural when distilled with 12 per cent HCl, and reacted with orcin and phloroglucin. The substance was not phytin; moreover, he concluded that wheat bran does not contain phytin and that the compound $\text{C}_{25}\text{H}_{55}\text{O}_{49}\text{P}_9$ is the only organic phosphoric acid existing in bran.

The study of the chemical properties of phytic acid was continued by Anderson⁶⁸ after he had observed that the organic phosphoric acids existing in cottonseed meal, oats, and corn yielded identical crystalline barium salts which differed in composition from the corresponding barium phytates, so-called. He then⁶⁹ resumed the study of the organic phosphoric acid compound of wheat bran. Rather's⁶³ claim that he had prepared a silver salt of an organic phosphoric acid of the formula $\text{C}_{12}\text{H}_{41}\text{O}_{42}\text{P}_9$ was held to be in error. Also he held that both Rather, and Patten and Hart had failed to make provision for eliminating inorganic phosphates in attempting to isolate the organic phosphoric acid. From the work reported at this time Anderson concluded that wheat bran contains more than one organic phosphoric acid, one of which, *viz.*, inosite monophosphate, was identified.

This was described in a later paper⁷⁰ as $\text{C}_6\text{H}_6(\text{OH})_5\text{O} \cdot \text{PO}(\text{OH})_2$. On cleavage with either dilute H_2SO_4 at 120° , or with 10 per cent ammonia at 150° in a sealed tube it decomposed into inosite and phosphoric

⁶⁵ *J. Biol. Chem.*, 11, 471 (1912).

⁶⁶ *Ibid.*, 12, 71 (1912).

⁶⁷ *Ibid.*, 12, 447 (1912).

⁶⁸ *J. Biol. Chem.*, 17, 171 (1914).

⁶⁹ *Ibid.*, 18, 425 (1914).

⁷⁰ *Ibid.*, 18, 441 (1914).

acid. No data were afforded as to the quantitative percentage in wheat bran.

It was very soluble in water, and insoluble in alcohol, ether, and the other usual organic solvents. In aqueous solution it gave no precipitate with barium hydroxide, or with barium or calcium chlorides. While silver nitrate formed no precipitate in aqueous solutions, the addition of alcohol to such a solution resulted in the precipitation of the silver salt. With lead acetate a heavy, white, amorphous precipitate formed which was readily soluble in dilute HCl or HNO₃.

Some years later Anderson⁷¹ reopened the discussion of the organic phosphorus compounds of wheat bran, and conceded that when inosite-penta-, -tetra-, or other lower inosite phosphoric acids are encountered, they have appeared in consequence of the partial hydrolysis of the inosite hexaphosphoric acid. The use of a stronger solution of HCl than was originally employed served to inactivate the enzyme phytase which was capable of hydrolyzing the hexaphosphate in 0.2 per cent HCl. Barium salts of inosite hexaphosphate were prepared from such extracts.

Phytin content of several samples of wheat and wheat flour was determined by Averill and King,⁷² using Heubner and Stadler's method. Calculated on an air-dry basis, three samples of wheat contained 1.16, 1.19 and 1.36 per cent of phytin (as C₆H₁₈O₂₄P₆), the last being hard spring (Marquis), and the first two soft wheats. A bran sample contained 4.53 per cent, which was the highest concentration encountered in the numerous materials that were analyzed, including nuts, soybeans, rye mill products, buckwheat and other seeds.

Of the eight flour samples, the range was from 0.66 to 1.28 per cent, and the average was 0.93 per cent. The description of the flours was not adequate for an appraisal of the relation between wheat type or composition and phytin content.

Salts of inositol phosphoric acid were found in all parts of plants by Bodalski,⁷³ particularly in seeds. In the fat-free wheat kernels he found 0.73 per cent, and the phosphorus content of the Ca-Mg inosital-phosphate from this source was 20.21 per cent. In recovering these salts Bodalski extracted the fine meal with 5 per cent acetic acid for six hours, filtered, and precipitated the inositol phosphates with ammonia. The precipitate was washed with an ethanol-ether mixture (1:1) and finally with ether.

Soft wheat of France was found by Feyte⁷⁴ to have a phytin phosphorus/total phosphorus ratio of about 0.76, a Syrian wheat 0.775, the

⁷¹ *J. Biol. Chem.*, **44**, 429 (1920).

⁷² *J. Am. Chem. Soc.*, **48**, 724 (1926).

⁷³ *Wiadomosci Farm.*, **58**, 683, 697 (1931); *Chem. Abs.*, **26**, 2767 (1932). Original not seen.

⁷⁴ *Compt. rend. acad. agr. France*, **19**, 925 (1933).

mean of three *durum* wheats 0.625. In flour the ratio was lower, being in the range of 0.30, and is more or less a function of the degree of extraction, increasing with the latter.

The phosphorus distribution in grains was determined by Webster.⁷⁵ Using Guerrant's microcolorimetric method for the determination of phospholipin phosphorus, Rather's phytin method, and Collison's inorganic phosphorus method, he obtained the following results with a sample of wheat, recorded in percentage of dry weight:

Total phosphorus	0.4274
Phytin "	.3033
Lipoid "	.0283
Inorganic "	.0210
Other "	.0748

From this it appeared that 71 per cent of the total phosphorus was in phytin combination and only 6.6 per cent as lipid phosphorus in the whole wheat which Webster analyzed.

Using Averill and King's modification of the Heubner and Stadler method for the determination of phytin, Andrews and Bailey⁷⁶ found about 86 per cent of the phosphorus of bran to be in that compound, while in germ the proportion was much lower. Their data, recorded in Table 81, suggest that germ contains more nucleo-protein phosphorus and lipid

Table 81. Distribution of Phosphorus in Constituents of Wheat Bran and Germ, as Reported by Andrews and Bailey.⁷⁶

Form of P compound	—Wheat bran—		—Wheat germ—	
	Found (%)	Part of total P (%)	Found (%)	Part of total P (%)
Total	1.646	100.0	1.244	100.0
Extracted by 2% HCl	1.61	97.8	1.004	80.7
Phytin	1.415	86.0	0.597	48.0
Lipoid	0.028	1.7	0.071	5.7
Other	0.203	12.3	0.576	46.3

phosphorus than the bran. Attention was called to an evident error in Averill and King's⁷² estimate of the phytin content of flour. In several instances they reported as high as 0.36 and 0.35 per cent of phytin phosphorus in flour. Since approximately 25 per cent of the ash of commercial patent flour can be assumed to be elemental phosphorus, it follows that if the phytin phosphorus reported by them was multiplied by 4, the flours would contain about 1.40 per cent of ash. The latter is several times higher than the percentage of ash reported by the manufacturers of the flours in question ($0.40 \pm$ per cent).

When Andrews and Bailey examined a sample of highly refined or

⁷⁵ *J. Agr. Research*, 37, 123 (1928).

⁷⁶ *Ind. Eng. Chem.*, 24, 80 (1932).

second middlings flour, they found the amount of phytin present to be too small to be detected. If all the phosphorus in this flour had been in phytin combination, nearly 0.32 per cent of phytin would have been present. This was well within the limits of sensitivity of the phytin method employed. Accordingly, it was evident that only a small proportion of the phosphorus in the endosperm of wheat occurred in the form of phytin.

In straight-grade flour representing 73 per cent of the wheat from which it was milled, Geoffroy ⁷⁷ found a total organic phosphorus content of 0.114 per cent. Of this organic phosphorus, 38 per cent was in combination with starch, 35 per cent in phytin, 14 per cent in lipoids, and 12 per cent in nucleoproteins. This represents practically all the phosphorus in flour, since the total phosphorus, as determined in the ash, was 0.118 per cent of the flour.

The ratio between the phosphorus and the total ash content of flour was believed by Berliner and Rüter to be so constant that a rapid colorimetric phosphorus determination was devised by them and described by Pelshenke ⁷⁸ which is intended to be substituted for an ash determination in determining the grade or degree of refinement of commercial flour.

Wheat germ was analyzed by S. Posternak and T. Posternak ⁷⁹ and found to contain the following types of phosphorus compounds in terms of solubility:

	Part of the germ	Per cent of the total P
1. Phosphorus, total	0.873	100
2. " in ether-soluble fraction	0.023	2.63
3. " in alcohol-soluble fraction	0.004	0.46
4. " in fraction extracted by 15% picric acid	0.761	87.17
5. " insoluble in these solvents	0.085	9.74
6. " mineral	Traces	

The phosphorus in fraction 5, insoluble in the several solvents, was presumed to represent triticonucleic acid.

When barium acetate was added to the picric acid extract, 89 per cent of the phosphorus appeared in the precipitate. This was purified and the prismatic crystals thus recovered corresponded to the formula $(C_6H_{12}O_{21}P_5 \cdot 2H_2O)_2Ba_5$. The free acid was slightly levorotatory, $(\alpha)_D = -3.92^\circ$, and on neutralization with soda it became $(\alpha)_D = -7.94^\circ$.

There appears to be a preponderance of tetraphosphoricinositol ether

⁷⁷ "Le blé, la farine, le pain," Paris, 1939.

⁷⁸ "Untersuchungsmethoden für Brotgetreide, Mehl und Brot," p. 33 (1938).

⁷⁹ *Compt. rend.*, 186, 261 (1928).

in wheat germ, which Posternak *et al.* suggest may be a primary product in the evolution of inositol-hexaphosphate.

In wheat germ (dried) Javillier and Colin⁸⁰ fractionated the phosphorus compounds into four groups, which included, in terms of phosphorus, the following substances and percentages: lipoid P, 0.120; phytin P, 0.567; nuclein P, 0.379, and mineral P, 0.289. The total phosphorus content of the germ was 1.356 per cent.

Lipoid phosphorus constituted from 9.4 to 12.7 per cent of the phosphorus in five samples of American hard winter wheat analyzed by Guerrant⁸¹ the average being 10.6 per cent. These wheats contained an average of 0.289 per cent of total phosphorus, and the lipoid phosphorus accordingly averaged 0.306 per cent of the total grain. This lipoid phosphorus was determined by extracting the seeds with an ether-alcohol mixture and then analyzing this extract for phosphorus. The wheats contained an average of 2.17 per cent fat or ether extract, and 1.88 per cent of total ash.

Zunini⁸² later reported 0.368 per cent of nuclein phosphorus, and 0.34 per cent of phytin phosphorus in wheat germ. Both of these percentages are lower than those of Andrews and Bailey, probably because of more non-germ impurities in the product examined by Zunini, but the latter's data confirm the former's observation that less than half of the phosphorus of wheat germ is in phytin combination.

About 79 per cent of the total phosphorus of wheat was found by Wiazownicka⁸³ to be in phytin combination, 26 per cent in nuclein and lecithin compounds, and 5 per cent as mineral or inorganic compounds. The precise determination of inorganic P_2O_5 was not easy, since phosphatase splits off phosphoric acid from several organic combinations at pH 3.8 to 7.6. There was some indication, to his mind, that when gliadin and calcium salts of phytic acid were extracted at the same time they tended to form combinations.

Phytin phosphorus represented a larger percentage of the total phosphorus in the whole wheat and the bran than in the flour, in the series of samples analyzed by Quagliariello.⁸⁴ (His analyses of the products of roller milling are included in Table 82.)

The relative proportion of the phosphorus in the nucleins of the flours was notably higher than in the other products, whereas the percentage of the phosphorus presumed to be in inorganic form in the shorts was remarkably high.

The percentage of phosphorus, as P_2O_5 , in the wheats of France, was

⁸⁰ *Bull. soc. chim. biol.*, 15, 1552 (1933).

⁸¹ *J. Agr. Research*, 35, 1001 (1927).

⁸² *Chim. ind. agr. biol.*, 11, 339 (1939).

⁸³ *Bull. Acad. Polonaise Sci. Lettres, (B)*, 107 (1933).

⁸⁴ *Quaderni nutriz.*, 6, 276 (1939).

Table 82. Distribution of Phosphorus in the Various Constituents of Roller Mill Products, as Reported by Quagliariello.⁸⁴

Product	% of Products	Ash (%)	Total P (%)	
Wheat		1.76	0.376	
Flour	70	0.48	0.115	
Clear flour	5	1.32	0.210	
White shorts	3	3.64	0.861	
Brown shorts	4	4.30	1.007	
Bran	18	5.90	1.308	

Product	Percentage of Total P in—				Ether Extract (%)
	Lecithin	Nucleins	Phytin	Inorganic (by diff.)	
Wheat	3.72	15.11	70.81	10.36	1.66
Flour	9.89	37.90	41.72	11.50	0.68
Clear flour	5.44	31.23	48.12	11.50	1.40
White shorts	1.67	10.35	49.42	38.56	3.30
Brown shorts	1.74	8.94	54.42	34.90	3.68
Bran	2.70	9.45	82.06	7.23	3.50

found by Feyte⁸⁵ to range from 0.80 to 1.41 per cent. Of the samples analyzed, 12 per cent contained less than 0.95 per cent, 79 per cent from 0.95 to 1.2 per cent, and 9 per cent contained over 1.2 per cent. Syrian wheats contained only 0.6 to 0.7 per cent P_2O_5 . The ratio of phytin P to total P in the wheat varied from 54 to 90 per cent, the average being 73 per cent. This ratio appeared to be higher in the soft than in the hard wheats.

Calcium salts of phytic acid were isolated from wheat and other "seeds" by Jarosz,⁸⁶ and the water solutions contained more mineral phosphoric acid than that prepared, (*i.e.*, dissolved) in 0.1 per cent nitric acid. Solutions of HCl and HNO_3 of 1 per cent concentration effected less hydrolysis of these phytates than more dilute, *i.e.*, 0.1 per cent concentrations, or than water alone, although even prolonged boiling in aqueous solutions nearly 3 days did not complete the hydrolysis.

White flour of 70 per cent extraction was found by Widdowson⁸⁷ to contain 51 mg of phytic acid per 100 g, National wheat meal of 85 per cent extraction 127 mg, and wheat meal of 92 per cent extraction 214 mg.

After comparing Allen's colorimetric method for the determination of phytate phosphorus with the Embden-Fetter gravimetric method and finding the results in good agreement, Pringle and Moran⁸⁸ applied the former method to samples of wheat and of certain English mill products with the results shown in Table 83.

Phytin phosphorus, calculated as P_2O_5 , constituted 0.13 per cent of bread flour analyzed by Di Stefano and Muntoni,⁸⁹ which contained 0.38

⁸⁵ *Compt. rend. acad. agr. France*, 19, 925 (1933).

⁸⁶ *Bull. Acad. Polonaise Sci. Lettres (B)*, 123 1933.

⁸⁷ *Nature* 148, 219 (1941).

⁸⁸ *J. Soc. Chem. Ind.* 61, 108 (1942).

⁸⁹ *Ann. chim. applicata* 28, 139 (1938).

Table 83. Total Phosphorus and Phytate Phosphorus in Wheat and Mill Products, as Reported by Pringle and Moran.⁸⁸

Description of sample	Total P (mg/100 g, dry basis)	Phytate P (mg/100 g, dry basis)	Phytate P in total P (%)
Manitoba wheat	381	274	72
Plate wheat	481	364	76
English wheat	451	328	73
Purified bran	1609	1439	89
Purified germ	1413	674	48
White flour (70% extraction)	121	38	31
White flour (75% extraction)	127	45	35
National wheat meal, 85% (average of 12 samples)	238	136.5	57

per cent of total P_2O_5 and 0.75 per cent of total ash, all determinations being reported on the dry basis. Thus about one-third of the phosphorus in this flour was assumed to be in phytin combination.

When 21 samples of wheat were examined for their phytin content by Young and Greaves⁹⁰ a large range was encountered, some varieties having twice as much phytin phosphorus as others. The lowest content was found in the Newkirk variety with 0.152 per cent, and the highest in Dicklow with 0.328 per cent. The percentage of the total phosphorus present in the phytin varied from 56.7 in Regal to 94.3 in Federation.

Between 90 per cent and 100 per cent of the total phosphorus in wheat bran is in the phytic acid salts, combined as a calcium-magnesium salt of the hexaphosphoric ester of inositol, in the opinion of Hays.⁹¹ Certain

Table 84. Analysis of Products of Two Milling Systems, Reported by Hays.⁹¹

	Fiber (%)	Ash (%)	Ca (%)	Total P (I) (%)	Phytic acid P (II) (%)	II/I (%)
<i>Milling System A (Simons System)</i>						
Wheat	1.76	1.56	0.046	0.35	0.203	57
72% ext. white flour	0.18	0.46	0.020	0.097	0.023	24
85% ext. wheat meal	0.91	0.90	0.041	0.189	0.11	59
Fine wheat feed	6.15	3.78	0.085	0.832	0.662	79
Bran	9.45	5.46	0.094	1.18	1.07	90
Germ	2.20	4.41	0.053	1.12	0.52	46
J scalper tails	5.35	3.60	0.069	0.86	0.60	70
M tails	2.15	2.70	0.037	0.54	0.46	85
<i>Milling System B</i>						
Wheat	2.10	1.52	0.057	0.334	0.215	64
75% ext. white flour	0.25	0.53	0.025	0.117	0.053	45
85% ext. wheat meal	1.00	0.97	0.042	0.204	0.135	66
95% ext. wheat meal	1.62	1.29	0.055	0.271	0.162	60
Fine wheat feed	6.80	3.96	0.084	0.86	0.73	85
Bran	10.30	5.54	0.096	1.21	1.17	97
Germ	2.05	4.53	0.051	0.94	0.49	52
M tails	3.55	3.39	0.080	0.742	0.715	96

⁹⁰ *Food Research* 5, 103 (1940).⁹¹ *Cereal Chem.* 19, 326 (1942).

white wheats appeared to have a lower phytic acid content than red wheats. In commercial wheat mill products the phytic acid phosphorus was directly proportional to the fiber content. Also the ratio of phytic acid to total phosphorus decreased in progressing from the fibrous stocks, such as bran, to the white flour. This is evident from the data in Table 84 when the shorter-extraction flours, *i.e.*, 72 and 75 per cent, are contrasted with the longer-extraction flours produced in the same mill. Also there appeared to be a linear relation between the fiber content and the phytic acid phosphorus in the instance of these mill products, except that in certain constituents of those products, notably germ and parenchymatous cellular tissue of the endosperm, the phytic acid phosphorus was in higher ratio to the fiber than in the pericarp.

Methods for the extraction of the phosphorus-containing constituents of cereals were studied by Hanke⁹² and the average of 25 determinations on 13 different commercial wheat flours was 0.047 per cent of phosphatide P in the dry material. This value is considerably higher than that commonly reported by other investigators.

IRON, COPPER, NICKEL, COBALT, ALUMINUM, ZINC AND MANGANESE

Using a ferric thiocyanate colorimetric method for the determination of iron, Peterson and Elvehjem⁹³ found 0.00091 per cent (=9.1 ppm) in patent flour, 0.00372 per cent (37.2 ppm) in whole wheat, the same quantity in the graham flour which he examined, and 0.00852 (85.2 ppm) in bran. These are in the same general range as the concentrations published a little earlier by Sullivan and Near.¹⁸

A comparison of three methods in the determination of iron in feeding stuffs is afforded in the study conducted by Fraps and Fudge.⁹⁴ The three methods included (I) that of Elvehjem and Hart involving removal from the solution of the ash of phosphoric acid as the molybdate, precipitation of the iron with KOH, solution of the washed precipitate and its color development as the sulfocyanate; (II) ferrocyanide method, involving addition of a potassium ferrocyanide-gum ghatti reagent to an acid solution of the ash, and comparing colorimetrically with an iron standard; (III) A.O.A.C. short method in which the color is developed directly by adding a large excess of sulfocyanate to the solution of the ash, thus eliminating precipitation of the iron, and avoiding fading and interference from phosphates.

These methods were applied to several wheat products, with the results shown in Table 85. Method I tended to give less concordant as well as lower results. Fair agreement was had between methods I and

⁹² *Mühlenlab.*, 10, 33 (1940).

⁹³ *J. Biol. Chem.*, 78, 215 (1928).

⁹⁴ *J. Assoc. Official Agr. Chem.*, 15, 307 (1932).

Table 85. Iron in Parts per Million, as Determined by Fraps and Fudge⁹⁴ in Several Cereal Products.

Product	Iron (ppm)		A.O.A.C. method
	Elvehjem and Hart method	Ferrocyanide method	
Wheat	57	75	
Wheat chop	108	127	130
" "	98	86	
Wheat gray shorts	221	149	150
" " "	150	148	169
" " "	149	131	154
Wheat brown shorts	107	101	96
" " "	149	160	161
" " "	203	197	
Wheat bran	258	265	263

II. The concentration of iron in the one sample of wheat reported here is higher than that commonly reported.

The whole wheat used by Rose, Vahlteich and Bloomfield⁹⁵ in their studies of hemoglobin regeneration contained 0.2 mg of iron per 6 g of grain. This is equivalent to $3\frac{1}{3}$ mg per 100 g, or 33.3 ppm.

In an extensive study of the ionizable iron in foodstuffs, as determined by the α , α -dipyridyl reagent to extracts of foods made by Shackleton and McCance,⁹⁶ certain cereal products were included. In white flour they reported 1.00 mg of total iron per 100 g, 93 per cent of which was ionizable; in white bread 1.07 mg, and in "whole meal" bread from two sources, 2.12 and 3.43 mg, with a rated availability of about 80 per cent.

In white and whole wheat bread Toscani and Retznikoff⁹⁷ found the following concentrations of iron in mg per 100 g of bread:

White bread	Max. 1.38	Min. 1.18	Ave. 1.27
Whole wheat bread	" 1.97	" 1.88	" 1.93

The values for white bread are higher than the Sherman⁹⁸ data (0.90 mg per 100 g), or the Rose⁹⁹ data (0.85 mg per 100 g), and nearly double the value of 0.67 mg per 100 g reported by Leichsenring and Flor.¹⁰⁰

The iron content of the ash from cereals and other materials was found by Hoffman, Schweitzer and Dalby¹⁰¹ to be higher when sodium hydroxide solution was added to the substance before incinerating it. Using this procedure they found the following percentages of iron, as milligrams per 100 g in certain cereal products: white flour (0.44 per cent

⁹⁵ *Proc. Soc. Exp. Biol. Med.*, 26, 322 (1929).

⁹⁶ *Biochem. J.*, 30, 582 (1936).

⁹⁷ *J. Nutrition*, 7, 79 (1934).

⁹⁸ "Chemistry of Food and Nutrition," 4th ed., New York, MacMillan, 1932.

⁹⁹ "A Laboratory Handbook for Dietetics," 3rd ed., 1929.

¹⁰⁰ *J. Nutrition*, 5, 141 (1932).

¹⁰¹ *Ind. Eng. Chem. (Anal. Ed.)*, 12, 454 (1940).

ash) 1.3; clear grade flour (0.72 per cent ash), 2.0; red dog flour (2.80 per cent ash), 10.7; bran, 12.7; whole-wheat flour, 5.0; germ, 10.0.

After discussing certain details essential to the accurate determination of the small proportions of iron in cereals, Andrews and Felt¹⁰² applied the dipyriddy reagent to the ash which had been previously subjected to acid hydrolysis to rid it of interfering pyrophosphate. No losses of added iron compounds were experienced when flour-iron mixtures were ashed at 575° overnight, and the resulting ash was taken up with hot HCl solution.

In the instance of the products from one roller mill, they were found to contain the percentages of iron shown in Table 86. In general the

Table 86. The Iron Content of the Products of Wheat Milling (basis of 13.5 per cent moisture), as Reported by Andrews and Felt.¹⁰²

Product	Ash (%)	Iron (%)	Iron in Ash (%)
Patent flour	0.41	0.00078	0.190
First clear	.82	.0019	.232
Second clear	2.34	.0062	.265
Red dog	3.70	.0113	.306
Shorts	4.18	.0127	.305
Bran	6.53	.0126	.193
Germ	4.14	.0086	.208
Wheat	1.65	.0037	.224

iron content tended to increase with the percentage of ash, reaching its highest level in the coarse products or feeds. There was no large difference in the iron content of the bran and shorts, however, which meant that the percentage of iron in the ash of the shorts was higher than in the ash of the bran.

Table 87. Iron Content of Products from Ten Different Mills, as Reported by Andrews and Felt.¹⁰²

Mill product	Average iron content, dry basis (mg/g)	Iron in ash	
		Average (%)	Range (%)
Patent flour	8.4	0.180	0.125-0.239
First clear	17.4	.219	.165- .250
Second clear	38.7	.258	.214- .290
Red dog	96.2	.302	.284- .337
Shorts	139.0	.281	.249- .335
Bran	146.2	.206	.187- .228
Germ	91.3	.192	.174- .210
Wheat	41.6	.232	.198- .262

In another series of samples representing ten mills, the iron content of which is shown in Table 87, the percentage of iron in the shorts

¹⁰² *Cereal Chem.*, 18, 819 (1941).

averaged more than that of the bran. The ratio of iron to total ash appears to be more variable in these samples examined by Andrews and Felt than in some of the other studies summarized in this monograph.

A summary of the published results of the analyses of wheat for its iron content was included in the paper by Free and Bing.¹⁰³ In addition to the data already recorded in the present monograph, the following analyses were reported:

Author	Citation	Total iron (mg/100 g)
Leach	"Food Inspection and Analysis," (1909)	3.06
Sherman	"Chemistry of Food and Nutrition," (1932)	5.00
Peterson and Elvehjem	<i>J. Biol. Chem.</i> , 78, 215 (1928)	3.72
Elvehjem, Hart and Sherman	<i>Ibid.</i> , 103, 61 (1933)	5.00
Saiki <i>et al.</i>	"Chemical Analysis of Food in Japan," Tokyo (1934)	3.5
Vahlteich, Rose and MacLeod	<i>J. Nutrition</i> , 11, 31 (1936)	3.33
Smith and Otis	<i>Ibid.</i> , 13, 573 (1937)	5.18
Aykroyd	"The nutritive value of Indian foods and the planning of satisfactory diets," Coonoor, India, (1937)	5.37
Goswami and Basu	<i>Indian J. Med. Res.</i> 25, 893 (1938)	5.5
Ranganathan	<i>Ibid.</i> , 25, 677 (1938)	3.97
Free and Bing	<i>J. Nutrition</i> , 19, 449 (1940)	3.94

Eleven samples of wheat, including soft red winter, hard red winter, hard red spring and *durum* wheats were in the collection of wheats analyzed by Free and Bing, and the range was from 2.90 to 4.87 mg of iron per 100 g of wheat, with an average of 3.94 mg. The "ionogenic" iron, as measured by the α, α' -dipyridyl method of Elvehjem, Hart and Sherman¹⁰⁴ averaged 81 per cent of the total iron. Actually the retention of wheat iron by test rats amounted to 50 per cent, but this was equal to the retention of the iron of ferric chloride.

The Council on Foods of the American Medical Association^{104a} accepted an average value of 18 mg of iron per pound (=3.9 mg/100 g) for whole wheat, and 4.5 mg/lb (= 1.0 mg/100 g) for white flour.

After citing the earlier results of Galippe's analysis of wheat, which showed 0.005 to 0.01 per cent of copper, Vedrödi¹⁰⁵ reported his analyses of spring wheat, in terms of cupric oxide content, as 0.11 per cent, and of winter wheat as 0.21 per cent. This would be equivalent to 0.088 per cent as elemental copper in the spring wheat, and 0.168 per cent in the winter wheat. Apparently these findings were challenged by K. B. Lehmann, who noted that they were higher by the order of ten times

¹⁰³ *J. Nutrition*, 19, 449 (1940).

¹⁰⁴ *J. Biol. Chem.*, 103, 61 (1933).

^{104a} *J. Am. Med. Assn.*, 116, 2849 (1941).

¹⁰⁵ *Chem. Ztg.*, 17, 1932 (1893).

than the analyses made by Tschirsch. Vedrödi¹⁰⁶ then reported additional analyses, as follows:

	Crop of 1894				Crop of 1895			
	As CuO (%)		As Cu (mg per kg)		As CuO (%)		As Cu (mg per kg)	
	max.	min.	max.	min.	max.	min.	max.	min.
Spring	0.080	0.030	630	190	0.030	0.025	230	190
Winter	0.090	0.010	710	80	0.086	0.025	680	200

These maximum findings are in the general range of those reported in 1893, and Vedrödi called attention to some of the possibilities of error in Lehmann's analytical techniques.

In an extensive study of the copper content of foods Guerithault¹⁰⁷ included wheat, flour, and wheat germ, which were found to contain 7.2, 2.0, and 48.0 mg per kg of fresh material (1 mg per kg = 1 ppm).

Copper content of American wheats received special attention at the hands of Webster and Jansma.¹⁰⁸ Wheats from 13 states contained an average of 6.0 mg per kg of material, ranging from 4.2 mg in an Oregon wheat to 8.7 in C.I. 1442 grown in California. The ash of these wheats contained an average of 345 mg of copper per kg, the average ash content being 1.78 per cent. Of the five Oklahoma wheats analyzed, the average copper content was 6.2 mg per kg. They felt that there was a trend in the direction of a lower copper content in the "northern" samples, although as a matter of fact no hard spring wheats from the northern Great Plains were included in the collection of wheat samples.

No correlation between the copper content of Utah soils, and of wheat grown on those soils was found by Greaves and Anderson.¹⁰⁹ Moreover, 16 varieties of wheat grown on the same soil contained from 5.6 to 16.7 parts of copper per million of grain, with an average of 9.7.

In their study of the copper content of foods Lindow, Elvehjem, and Peterson¹¹⁰ found the following quantities, in terms of mg per kg: patent flour, 1.7; graham flour, 4.9; wheat bran, 11.7; and wheat germ, 12.7. The study was extended by Elvehjem and Hart,¹¹¹ and these additional data are selected from their tabulation: wheat, 7.8; bran, 16.4; germ, 9.0; wheat gluten, 17.2; and wheat middlings, 12.1. It is thus evident that a substantial relative variation in copper content was encountered among these products.

Nickel and cobalt were determined in "froment (graine)," and "froment (son)" by Bertrand and Mokragnatz¹¹² with the following results in fresh material:

¹⁰⁶ *Ibid.*, 20, 399 (1896).

¹⁰⁷ *Bull. soc. hyg. aliment.*, 15, 386 (1927).

¹⁰⁸ *Science*, 70, 174 (1929).

¹⁰⁹ *J. Nutrition*, 11, 111 (1936).

¹¹⁰ *J. Biol. Chem.*, 82, 465 (1929).

¹¹¹ *Ibid.*, 82, 473 (1929).

¹¹² *Bull. soc. chim. (4)*, 37, 554 (1925).

	Ash	Nickel (%)	Cobalt (%)
Froment (graine)	(3.4 in 900 g)	0.000030	0.000001
" (son)	(5.3 in 985 g)	0.000035	0.000001

A large number of foods were later analyzed by Bertrand and Mokragnatz¹¹³ to determine their nickel and cobalt content; among these were included wheat and wheat bran:

	mg/kg of dry material	
	Nickel	Cobalt
Entire wheat kernel	0.35	0.012
Wheat bran	0.39	0.011

There was a surprisingly small difference in the amount of these two metals present in the entire grain and in the bran milled from it.

Bertrand and Levy¹¹⁴ also determined the aluminum content of wheat and mill products with the following results, in terms of milligrams of aluminum per kilogram of dry material:

Entire wheat	4.0 mg	Fine bran	10.2 mg
Flour I	0.95	Fine middlings	12.8
Flour II	1.9	Coarse middlings	5.1
Flour III	1.2	Germ	143.
Coarse bran	12.7		

Unlike nickel and cobalt, there was a substantially higher concentration of aluminum in the bran than in the whole wheat. The relatively large percentage in the fine middlings was due to a much larger proportion of aluminum in the ash of that product.

The zinc content of numerous foods was determined by Bertrand and Benzon.¹¹⁵ In the entire grain they found 18.7 mg per kg, in straw, 21.8 mg, and in bran 38.0 mg. There was no large difference in the zinc content of wheat, oats, corn (maize) and barley.

A turbidometric ferrocyanide method for the determination of zinc was developed by Birckner¹¹⁶ which he applied to a wide range of food materials including wheat. In four wheat samples he found the following quantities, reported as milligrams per kg of fresh substance:

Dietz, Virginia.	84.8
Fultz, Virginia	44.7
Early Baart, Pacific Coast	26.1
Marquis, South Dakota	47.4

A sample of bran was also analyzed and found to contain 139.2 mg per kg of Zn.

In the early stages of his studies upon the possible physiological role

¹¹³ *Bull. soc. chim.* (4), 47, 326 (1930).

¹¹⁴ *Ibid.*, 49, 1417 (1931).

¹¹⁵ *Bull. soc. hyg. aliment.*, 16, 457 (1928).

¹¹⁶ *J. Biol. Chem.*, 38, 191 (1919).

of manganese in plants, McHargue¹¹⁷ found 0.0055 per cent of that element in wheat flour, and 0.0194 per cent in wheat bran.

The concentration of manganese in the developing wheat grain tended to diminish as maturity was approached in the studies conducted by Javillier and Imas.¹¹⁸ On June 23, 1925 the grain contained 4.9 mg of Mn per 100 g dry matter, while on July 27 it had fallen to 3.4 mg. In a collection of 17 ripened wheats, the Mn content ranged from 2.6 to 4.8 mg per 100 g, with an average of 4.1 mg. The ash of these wheats contained from 119 to 238 mg of Mn per 100 g, with an average of 204 mg. In the products of milling, the straight grade flour contained only 0.54 mg of Mn per 100 g while in the coarser by-products it was in the range of 13 mg. The germ had the highest concentration, 22.5 mg. In the same series of mill products, these investigators¹¹⁹ found 0.91 mg of zinc in each 100 g of straight flour, with increasing amounts in the coarser by-products, reaching 10.4 mg in the bran, while the germ contained 19.9 mg (on dry matter basis in all instances). Five samples of whole wheat were analyzed and found to contain from 1.6 to 2.9 mg of Zn, averaging 2.0 mg.

Wide varieties of plant and animal materials were subjected to analysis for the determination of their manganese content by Lindow and Peterson,¹²⁰ who employed the periodate method of Willard and Greathouse. In whole wheat they found 37 mg per kg of dry sample, and in bran 101 mg.

Manganese, as determined in wheats by the A.O.A.C. periodate method, was found by Davidson¹²¹ to range from 0.0034 to 0.0086 per cent, reported as Mn_3O_4 , the average being 0.0066 per cent. In the instance of flour, shorts, and bran, the Mn_3O_4 content was 0.0027, 0.0465, and 0.0432 per cent respectively. The difference between bran and shorts was small despite the relatively large difference in total ash content, which was 7.27 per cent and 4.07 per cent in these two mill products.

A method for the determination of manganese was developed by Newcomb and Sankaran¹²² who applied it to numerous foods including wheat, flour, and bread. In Punjab wheat they found 43 mg per kilogram (parts per million), in American flour 12 mg, and in white bread 3 mg.

Manganese content of numerous foods of all types was determined by Peterson and Skinner.¹²³ In patent flour they found 4.0 mg per kg; in graham flour 42.8 mg; in bran flakes (edible) 49.9 mg.

¹¹⁷ *J. Am. Chem. Soc.*, **36**, 2532 (1914).

¹¹⁸ *Compt. rend. acad. agr. France*, **12**, 721 (1926).

¹¹⁹ *Ibid.*, **12**, 727 (1926).

¹²⁰ *J. Biol. Chem.*, **75**, 169 (1927).

¹²¹ *Cereal Chem.*, **6**, 128 (1929).

¹²² *Indian J. Med. Research*, **16**, 788 (1928).

¹²³ *J. Nutrition*, **4**, 419 (1931).

Flour of type 75 per cent was found by Bruere¹²⁴ to contain 0.68 mg of manganese per 100 g flour (dry basis), while a second or lower grade of flour containing 1.49 per cent of ash had a manganese content of 2.58 mg per 100 g (dry basis).

Wheat endosperm was found by Bruere¹²⁵ to contain 0.0007 per cent of manganese; the envelope 0.013 per cent and the germ 0.019. Using the proportions of 83:15.5:1.5 of these tissues, the weighted mean thus became 0.00288 per cent in the entire grain. On analysis of domestic (French) wheat they found 0.0025-0.0039 per cent, the low figure being the percentage in very plump, heavy kernels, the higher percentage that in less plump grain.

The endosperm was then divided into three zones, designated as (I) central, (II) median, and (III) peripheral, and representing percentages of 50, 35, and 15 respectively of this tissue. The manganese content of these zones in percentages was (I) 0.0004, (II) 0.0008, and (III) 0.0014. Thus the concentration of Mn was much lower in the central or interior portion of the endosperm than in the outer or peripheral portion.

Bruere also fractionated the envelope of the kernel, and found 0.0065 per cent of manganese in the external layers, and 0.017 per cent in the internal layer. He emphasized that the latter is also rich in lipids whereas the former is cellulosic in nature.

Coefficient of variation in four series of 25 samples each of Argentine wheats in the instance of manganese content of samples analyzed by Albizzati and Carradó¹²⁶ averaged 24 per cent. The arithmetic mean of the manganese content was 0.0049 per cent and the range from 0.0028 to 0.0090. From the general appearance of the arrays involving the plotting of Mn against total ash content within a variety, it would appear that the correlation between these two variables was not very high.

The manganese content of seven samples of American wheat was found by Hoffman, Schweitzer and Dalby^{126a} to range from 20.0 to 46.6 $\mu\text{g/g.}$ and to average 35.7 $\mu\text{g/g.}$ This is about 27 per cent lower than the value reported by Skinner and Peterson^{126b} which was 49.0 $\mu\text{g/g.}$ Hoffman, *et al.*, found 104.0, and 139.0 $\mu\text{g/g}$ of manganese in two samples of bran, 77.0 and 55.0 in red dog, 82.0 and 102.0 in germ, 8.5, 9.1, and 18.2 in clear flours, and 3.1 to 6.1 (average 4.2) in patent flour. A graph is presented which demonstrates a high correlation between the ash content of a collection of flour mill streams and the manganese and iron content.

¹²⁴ *Ann. fals.* 27, 150 (1934).

¹²⁵ *Compt. rend.*, 198, 504 (1934).

¹²⁶ *Ann. Soc. Cient. Argentina*, 121, 97 (1936).

^{126a} *Cereal Chem.*, 20, 328 (1943).

^{126b} *J. Biol. Chem.*, 79, 679 (1928).

The concentrations of lead, tin, and silver in wheat and certain mill products were determined spectrographically by Kent.¹²⁷ In terms of milligrams per 100 g, the lead content of wheat averaged 0.10, of flour 0.09, of germ 0.15, of bran 0.30, of "beeswing" (aspirated from bran) 0.48. The concentrations of tin, in mg/100 g of the same products were 0.09, 0.12, 0.04, 0.16, and 0.39 respectively, which values are in the same general range as those of lead with the exception of the germ. Silver in these products represented 0.04, 0.03, 0.08, 0.09 and 0.14 mg/100 g respectively or about one-third to one-half that of tin and lead.

POTASSIUM, MAGNESIUM, AND CALCIUM

Wheats grown in nutrient solutions by McCalla and Woodford¹²⁸ exhibited some response in terms of potassium content when a low K solution was used in feeding the plants.

In a series of twenty American hard and soft wheats collected from a wide range of area, Sullivan and Near¹²⁹ found the elements in the ash to vary in percentages through the following range: magnesium, as MgO, 11.47 to 16.30; calcium, as CaO, 2.06 to 4.47; phosphorus, as P₂O₅, 44.24 to 54.42; potassium, as K₂O, from 25.45 to 33.01; and the total ash from 1.70 to 2.23 of the wheat itself. Sullivan and Near felt that the magnesium content of the ash was related to quality, since most of the wheats having an ash containing more than 15 per cent of magnesium oxide were rated as "good" or "very good." Moreover, all of the "very poor" flours came from wheats in which the ash contained less than 13.5 per cent of MgO.

Incidental to an extensive discussion of the role of magnesium in the life of plants, Javillier¹³⁰ reported the percentage of this element in many foods, and plant parts. In wheat kernels he found 150 milligrams per 100 g of dry substance, and 7.236 per cent of the ash was magnesium. The percentages in certain products of milling are shown in Table 88.

Table 88. Magnesium in the Products of Milling as Determined by Mlle. Cremien and Reported by Javillier.¹³⁰

Material	Yield in percentage of wheat	Magnesium (%)
Farina	3.23	0.0085
White flour	69.62	0.022
Low grade flour	5.82	0.120
Gray shorts	2.04	0.301
Coarse shorts	5.21	0.327
Fine bran	2.63	0.454
Coarse bran	9.99	0.513
Germ	0.02	0.308

¹²⁷ *J. Soc. Chem. Indus.*, 61, 183 (1942).

¹²⁸ *Can. J. Research (C)*, 13, 339 (1935).

¹²⁹ *J. Am. Chem. Soc.*, 49, 467 (1927).

¹³⁰ *Bull. soc. chim. biol.*, 12, 709 (1930).

Unfortunately the total ash was not recorded in this instance and so it is not possible to determine the relative variability of the ratio of Mg to other ash constituents.

Aleurone cells were identified in flour suspensions by Berliner and Rüter¹³¹ by treating with commercial colloidal iron hydroxide. In a few seconds ring-form precipitation zones appeared which could be identified under the microscope using a magnification of about 250x.

On treating wheat products in alkaline solution with Chinalizarin, Titangelb, and Azoblau dyes, Broda¹³² effected a characteristic coloration of aleurone cells. This color reaction was attributed to the presence of magnesium compounds in the globoides of the cells in question.

The percentage of calcium found in a collection of 40 milling wheats by Zalar¹³³ was lower than that reported from most of the other sources. The calcium content ranged from 0.023 to 0.045, and the average was 0.033 per cent. Of these samples 30, or 75 per cent contained between 0.30 and 0.40 per cent of Ca.

Zalar also analyzed a series of typical flour mill products for their calcium content. As shown by his data in Table 89 the concentration

Table 89. Calcium Content of Flour Mill Products as Reported by Zalar.¹³³

Mill product	Yield of product, in percentage of cleaned wheat	Ash in product (dry basis) (%)	Calcium in product (dry basis) (%)	Calcium in ash (%)	Distribution of total calcium in mill products (%)
Wheat	100.00	1.86	0.040	2.15	100.00
Patent flour	62.30	0.45	0.018	4.00	25.1
First clear flour	8.67	0.88	0.025	2.84	4.9
Second clear flour	3.92	2.46	0.051	2.07	4.5
Red dog	2.37	4.01	0.086	2.14	4.7
Shorts	9.12	5.87	0.122	2.08	25.0
Bran	13.85	7.17	0.114	1.59	35.5
Germ	0.22	5.20	0.058	1.11	0.3

in the patent flour was low, but owing to the large yield of patent flour, it contained one-quarter of the calcium in the total products of the mill. Bran and shorts contained most of the calcium, due to the high relative concentration in those products. The ratio of calcium to total ash was quite variable, and tended to decrease in progressing from the highly refined products, such as patent flour, to the coarser products, such as bran.

The Council on Foods of the American Medical Association¹³⁴ accepted an average value of 240 milligrams of calcium per pound (= 53

¹³¹ *Z. ges. Mühlenw.*, 6, 180 (1930).

¹³² *Z. Untersuch. Lebensm.*, 70, 470 (1935).

¹³³ Private communication, July 1941.

¹³⁴ *J. Am. Med. Assn.*, 116, 2849 (1941).

mg/100 g) for whole wheat and 72 mg/lb. (= 16 mg/100 g) for white flour.

Sodium and potassium were determined in a number of food materials by Bertrand and Rosenblatt,¹³⁵ and wheat was found to contain 0.0015 per cent of the former (dry basis), and 0.3250 per cent of potassium. In the stem and glumes the concentration was much higher, on the dry basis the percentages being 0.12 Na, and 0.67 K. Note further that the ratio in the latter was 1:5.56, whereas in the grain it was 1:215.

¹³⁵ *Compt. rend.*, **186**, 200 (1928).

Chapter 13

The Halogens, Sulfur and Selenium

THE HALOGENS

The total chlorine content of unbleached flours was found by Utt¹ to range from 442 to 576 parts per million with an average of 515 ppm. When the same flours were bleached with chlorine, the total chlorine was increased in every instance, the average increase being about 300 ppm. Among the several chlorine-bleached flours examined by Utt, the range in total chlorine content was from 648 to 972 pp million, the average being 823 pp million. It is evident that the difference between the highest percentage of chlorine in an unbleached flour, and the lowest percentage in a bleached flour is not very large, being the difference between 576 pp million and 648 pp million.

While some chlorine is found in the ash of wheat and its products, most of this element is lost in the process of ignition as shown by Sullivan and Howe.² In wheat, patent flour, clear grade flour, and bran, they found 0.0546, 0.0507, 0.0601, and 0.0387 per cent respectively.

The chlorine content of the ether extract of natural flour was low in the majority of the analyses reported to Rask³ in his collaborative studies, ranging from none to 27 pp million of the flour, and averaging 16.6 ppm. This was significant in view of the fact that bleaching the flour with chlorine increased the chlorine content of the fat to the equivalent of an average of 93.7 ppm based upon the flour.

In wheat from a goiterous region McClendon and Hathaway⁴ found 1 milligram of iodine per ton (metric) of desiccated food, while in wheat from two non-goiterous areas (Connecticut and Maine) they found 3.5 mg and 8 mg respectively.

A sample of wheat ground in an experimental mill was found by them⁵ to yield products containing varying concentrations of iodine. Their data are recorded in Table 90. It appears that those products which are characterized by the highest concentrations of fiber, and minerals, also contain the most iodine. Other data, included in the

¹ *J. Ind. Eng. Chem.*, 6, 908 (1914).

² *Cereal Chem.*, 6, 396 (1929).

³ *J. Assoc. Official Agr. Chem.*, 6, 68 (1922).

⁴ *Proc. Soc. Exp. Biol. Med.*, 21, 129 (1923).

⁵ *J. Am. Med. Assoc.*, 82, 1668 (1924).

Table 90. Iodine Content of Wheats, and of Mill Products as Reported by McClendon and Hathaway.⁵

Description of sample	Iodine Mg per metric ton	Distribution in products (%)
Wheat from Storrs, Conn.	4	
Wheat from Edgecomb, Maine	9.3	
Wheat from Minnesota	6.6	
Straight flour from Minnesota wheat	3.5	35.9
Bran from Minnesota wheat	15.5	49.8
Shorts from Minnesota wheat	9.6	13.7
Red dog from Minnesota wheat	3.7	0.6

same table, lend further emphasis to the influence of environment upon the iodine content of wheat.

In a discussion of the iodine problem Glimm⁶ found 72.3 micrograms of that element in a kg of spring wheat. The wheat kernels were then cut crosswise into three portions, (1) the germ end, (2) the middle grain, and (3) the kernel tip, and these contained (1) 47.5, (2) 36.9, and (3) 425 micrograms of iodine per kg respectively. Thus, while the iodine content of the germ appeared to be significantly higher than the middle grain, it was much lower than the kernel tip.

This study was continued by Glimm⁷ and in five wheat samples similarly divided into three portions as before, he found an average of (1) 91.6, (2) 48.1, and (3) 208.2 micrograms per kilo of material. Much the same general relations were encountered in like portions of rye and barley kernels.

Five entire wheat kernels, chiefly from the Danzig area, contained from 54.4 to 84.7 micrograms of iodine per kg of grain. Rye grown in the same areas appeared to contain more iodine than wheat, barley and oats, the last three being quite similar in iodine content.

In three samples of winter wheat grown at Sprakel, Dreisborn, and Denz (Westfalia, Germany), the iodine content of the grain was found by Balks⁸ to be 78, 65 and 58 μg per kg of grain, respectively. The Denz soil appeared to contain less iodine than the soil of the other two localities. Spring wheat grown at Sprakel contained slightly more iodine than the winter wheat, namely 87 μg per kg of the grain.

In Japanese spring wheat Shirahama and Shimizu⁹ found 25.7 μg of iodine per 100 g dry matter, and in winter wheat 28.5 μg . This is 0.257 and 0.285 μg per g respectively, which is equivalent to that number of parts per million. Consequently this is substantially greater than the 0.006 ppm reported by McClendon and Hathaway.^{4,5}

⁶ *Biochem. Z.*, **219**, 148 (1930).

⁷ *Ibid.*, **243**, 88 (1931).

⁸ *Z. Untersuch. Lebensmittel.*, **71**, 76 (1936).

⁹ *J. Agr. Chem. Soc. Japan*, **8**, 527 (1932); *Chem. Abst.*, **26**, 4388 (1932). Original not seen.

A method for the determination in flour of materials which contain chloramine has been described by Pelschenke.¹⁰

The chlorine content of 32 Hungarian flour samples, as determined by Pap¹¹ varied between percentages of 0.0602 and 0.0738. Detection of chlorine treatment was better applied to the fat of the flour, rather than to the entire flour itself. Thus Pap found that 38 per cent of the chlorine used in treating flour was found in the fat when the latter was extracted with petroleum ether.

The bromine and chlorine content of a large number of foods was determined by Damiens and Blaignan.¹² They found 0.21 mg of bromine per 100 g of dry wheat, 0.09-0.12 in dry flour; 0.07-0.075 mg of chlorine per 100 g of dry wheat, 0.043-0.077 in dry flour. Thus in wheat the bromine content was about three times that of chlorine; in flour the ratio was between 1:1.2, and 1:2.7.

Bromine in wheat and flour was determined by Ibanez,¹³ who employed a method designed to minimize losses in ignition of the alkaline-alcohol digest. The final measurement was made colorimetrically in a chloroform extract of a preparation made after application of the Deniges-Chelles reaction to an aliquot of an aqueous extract of the ash. The wheats examined contained 0.0010 to 0.0075 g combined Br per kg and all the flours contained between 0.003 and 0.004 g per kg with the exception of two varieties (Casilda, and Santa Fe), which contained 0.0055 g per kg.

The Br content of flours and breads was determined by Ford, Kent-Jones, Maiden and Spalding.¹⁴ In parts per million, the content of several flours ranged from 2.4 to 7.7, averaging 4.9 ppm. In white-bread crumb with a normal moisture content, and from untreated flour, the Br content ranged from 1.6 to 5.4 ppm, averaging 3.3 ppm. One sample of whole-meal bread contained 6.5 ppm.

SULFUR

A fairly constant ratio of sulfur to protein was observed in wheat, patent flour, clear grade flour, and bran by Sullivan and Howe,² who reported 0.1943, 0.1648, 0.2104, and 0.2547 per cent of S in these four materials respectively. Glutens prepared from patent, and from clear grade flours contained 0.9724, and 0.9286 per cent of sulfur respectively. This led them to conclude that most of the sulfur of wheat and its prod-

¹⁰ *Untersuchungsmethoden für Brotgetreide, Mehl und Brot*, p. 245. 1938.

¹¹ *Kísérlet, Közlemények*, 31, 480 (1928).

¹² *Compt. rend.*, 193, 1460 (1931).

¹³ "Bromine in Flours and Wheats". (Pamphlet). 12 pp., Argentina, (1936); *Chem. Abst.*, 31, 6352 (1937). Original not seen.

¹⁴ *J. Soc. Chem. Ind.*, 59, 177 (1940).

ucts is combined organically in the protein, presumably as a component of cystine.

In a collection of wheats representing a wide area of wheat production Guillemet and Schell¹⁵ found from 0.1 to 0.2 per cent of total sulfur. They comment that if sulfo-ethers are present it is in such small quantities as to be insignificant. Mineral sulfates do not appear to constitute more than 4 to 5 per cent of the sulfur present in wheat. Most of the sulfur appears to be in protein combination. To test the relationship between sulfur and protein content of wheats they computed the S/N ratio in the instance of a collection of 13 samples ranging from 1.35 per cent to 2.56 total nitrogen content. The S/N ratio ($\times 100$) was not constant, but varied from 6.76 to 8.76. On scanning their data, there appears to be a tendency toward a descending S/N ratio with increasing nitrogen (=protein) content, but this relationship is not regular or precise.

Incidentally these thirteen wheats contained from 0.118 to 0.173 per cent of sulfur, with an average of 0.150 per cent.

The ratio of phosphorus to sulfur in various mill products was found by Bertrand and Silberstein,^{16, 17} to be quite variable. As shown by their data, recorded in Table 91, the highest S/P ratio was in the flour, *viz.*

Table 91. Ash, Sulfur, and Phosphorus Content of Wheat and Its Mill Products, and Gluten, as Reported by Bertrand and Silberstein¹⁶

Material	Ash (%)	On dry matter basis	
		Sulfur (%)	Phosphorus (%)
Wheat	1.92	0.161	0.476
Flour, 68 per cent extraction	0.72	0.190	0.183
Coarse bran	7.19	0.123	1.368
Fine bran	6.93	0.131	1.485
Coarse shorts	5.71	0.167	1.239
White shorts	5.03	0.206	1.200
Germ	5.55	0.231	1.298
Crude gluten	0.94	0.926	0.328
Gluten soluble in 70 per cent ethanol		0.349	0.505
Gluten insoluble in 70 per cent ethanol		1.002	0.304
			S/P (%)
			0.338
			1.039
			0.090
			0.099
			0.135
			0.172
			0.178
			2.824
			0.691
			3.296

1.039, the lowest in the coarse bran, 0.090. When the samples were arranged in order of increasing ash content there was a fairly definite trend in the direction of a decreasing S/P ratio.

Crude gluten was then recovered from the flour and analyzed for the same elements. A portion of this gluten was dried, ground, and digested several days with 70 per cent ethanol. The soluble portion was then analyzed, as was also the insoluble residue. Unfortunately Bertrand

¹⁵ *Compt. rend.*, 196, 1052 (1933).

¹⁶ *Compt. rend.*, 197, 285 (1933).

¹⁷ *Ann. Inst. Pasteur*, 51, 669 (1933).

and Silberstein did not indicate the proportion of the total gluten that appeared in each protein fraction. The analysis as recorded in Table 91 indicates that the alcohol-soluble fraction was low in sulfur, and high in phosphorus. The latter characteristic might be explained on the basis that phosphatides such as lecithin present in the crude gluten would be extracted by alcohol. The occasion for the low sulfur content is not apparent, however, unless it be assumed that the alcohol-soluble proteins (commonly termed "gliadin") are lower in cystine and other sulfur-containing amino acids than the alcohol-insoluble gluten proteins.

The relatively high sulfur content of the gluten affords a basis for interpreting the data derived from the analysis of the mill products, since it appears possible that much of the sulfur of these materials is present in the proteins which they contain. Phosphorus, on the other hand, is present in considerable amounts in non-protein constituents, such as phytin, in bran and shorts.

The sulfur and phosphorus content of a collection of plants cultivated on the same soil was determined by Bertrand and Silberstein,¹⁸ and in the wheat kernels they found 0.26 per cent of sulfur and 0.41 per cent of phosphorus (dry basis). The S/P ratio accordingly was 0.633, which placed wheat in the lower levels of this ratio in the list of some thirty-odd food plants. As might be expected, the cruciferae, including mustard, radish, and cabbage, were high in sulfur content; in fact, the percentage of the latter varied over a much wider range (twelvefold) than the percentage of phosphorus (about threefold).

A coefficient of correlation of $r = +0.80$ between sulfur and protein content of Utah wheats was observed by Greaves and Bracken¹⁹ in a series including 6 spring, and 15 winter wheats. The average ratio between these two elements was $N/S = 14.5$, with an average of 0.18 per cent of sulfur, and 2.61 per cent of nitrogen. They concluded that the evidence points to the existence of the sulfur in organic form.

Cystine-sulfur in wheat was found by Gubler and Greaves²⁰ to constitute about half of the total sulfur. Thus in three series of wheat samples the averages of the cystine S: total S ratio were 0.502, 0.553, and 0.534 respectively. The cystine-sulfur content was 0.099 per cent in the first series, 0.096 in the second series, and 0.095 in the third series, and 0.0967 for the 21 samples. Applying these averages, it appears that the total sulfur content of these samples must have been 0.180 per cent. The cystine content averaged 0.363 per cent, and ranged from 0.338 per cent to 0.405 per cent.

¹⁸ *Compt. rend.*, 201, 1449 (1935).

¹⁹ *Cereal Chem.*, 14, 578 (1937).

²⁰ *Food Research*, 7, 405 (1942).

Spring wheat plants grown at Versailles, France, were found by Maume and Bouat²¹ to contain more sulfur than winter wheats, at the time of the appearance of the awns, and also at flowering. The same order of relationship was also observed by Maume and Dulac²² insofar as the sum of N, P, and K are concerned.

Little difference in the sulfur content of white and "wholemeal" flour was disclosed by the analyses conducted by Masters and McCance²³ who reported 108.5, and 123.5 mg per 100 g respectively.

A sulfur-containing material was recovered from a petroleum ether extract of flour by Balls and Hale²⁴ to which more extended reference is made in the section on lipoids.

SELENIUM

A series of bulletins was published by Byers,^{25, 26} Byers, Miller, Williams, and Lakin,²⁷ and Williams, Lakin, and Byers²⁸ in which the occurrence of selenium in certain American soils and related matters was discussed. The early studies of "alkali disease" instituted by Franke, and of the tracing of the toxicity of wheat to the presence of selenium that was done by Robinson is related in the first paper in the series. The data presented in these four bulletins relate chiefly to the selenium content of soils. In the first bulletin, reference is made to the examination of several thousand soil samples that had been analyzed, taken from an area of a half million acres, all of which contained selenium in quantities ranging from a trace to about 40 pp million. The selenium content of wheat plants was not invariably a function of the concentration of selenium in the soil, however. Thus in the instance of one soil in Lyon County, South Dakota, which contained 2.5 ppm of selenium, the wheat plants grown on it contained 45 ppm. Another field in the same section, with soil containing 3 ppm of selenium produced wheat plants containing 10 ppm; a third field in that section with soil containing 3 ppm produced wheat plants containing only 0.5 ppm. On a field in Albany County, Wyoming, having a soil containing 2.5 ppm, the wheat plants contained 45 ppm and the ripened grain only 2 ppm. Incidentally, by way of emphasis upon the facility with which certain species of plants will accumulate selenium a collection of *Astragalus bisulcatus* from the Albany County wheat field contained 1,110 ppm of Se.

On the other hand, limonitic concretions from a Niobrara formation

²¹ *Compt. rend. acad. agr. France*, 23, 426 (1937).

²² *Ibid.*, 23, 89 (1937).

²³ *Biochem. J.*, 33, 1304 (1939).

²⁴ *Cereal Chem.*, 17, 248 (1940).

²⁵ *U. S. Dept. Agr. Tech. Bull.*, 482 (1935).

²⁶ *U. S. Dept. Agr. Tech. Bull.*, 530 (1936).

²⁷ *U. S. Dept. Agr. Tech. Bull.*, 601 (1938).

²⁸ *U. S. Dept. Agr. Tech. Bull.*, 702 (1940).

were mixed with a good quality soil by Beath, Eppson, and Gilbert²⁹ to give a composite soil containing (1) 13.8 pp million of selenium in the first instance, and (2) 65 ppm in the second instance. Wheat seedlings were grown on both, and the seedlings from (1) contained 4 ppm, from (2) 25 ppm. Thus the ratio between the selenium content of the wheat plants was not far different from that of the selenium content of the soils on which they were grown. In these instances, the type of selenium compound supplied was identical in both instances, and the nature and proportion of sulfur compounds was also much the same, which is not true when natural soils are compared.

Selenium was found by Moxon, Olson, Searight and Sandals³⁰ to occur in all Cretaceous formations in South Dakota from the Dakota sandstone up through the Pierre formation. In the Pierre formation, the concentration varies greatly in the different members, being high in the Sharon springs, locally high in the Mobridge, and generally low in the Sully and Virgin Creek. There appear to be high concentrations where the (Mobridge) member is thin, low concentrations where it is thick.

In another publication Moxon³¹ emphasized that the selenium content of plants grown upon seleniferous soils depends upon several factors, only one of which is the amount of total Se present. The form of selenium compounds is also important. It appears that in the natural sequence of soil formation from seleniferous rocks, converter plants such as Astragali, Aster, and others possessed the faculty of absorbing compounds of selenium that are not available to the grasses, including wheat. When these converter plants die and decay they return to the soil forms of selenium compounds which are available to grass plants. Thus these converter plants play an important role in the selenium problem and the areas which produce toxic crop plants are generally infested with converter plants.

In a later bulletin, Moxon, Olson, and Scaright³² trace the selenium cycle from seleniferous igneous rocks and rock wastes to the oceans, from which certain marine deposits were laid down as sedimentary rocks that became soils; and thence through converter plants selenium compounds became available to crops, whence they entered the diet of animals. The selenium content of various sedimentary rocks and the factors determining the selenium content of soils are traced very effectively and in considerable detail. Wheat plants grown in the field were analyzed at various stages of growth, and it was found that the concen-

²⁹ *Wyo. Agr. Exp. Sta. Bull.*, 206 (1935).

³⁰ *Am. J. Botany*, 25, 794 (1938).

³¹ *So. Dak. Agr. Exp. Sta. Bull.*, 311 (1937).

³² *So. Dak. Agr. Exp. Sta. Tech. Bull.*, 2 (1939).

tration in the stems and roots diminished progressively as the plants approached maturity, and that the heads of these particular plants contained 16 ppm of Se at maturity (dry basis).

A convenient survey of the selenium problem as revealed by the literature up to 1936 was published by Trelease and Martin.³³ The earlier experiences with toxic loco weeds, the erroneous assumptions concerning the role of alkalis, and particularly of barium, and the unfolding of the present views involving the toxicity contributed by the presence of selenium are reviewed. These authors indicate that they are not certain that the toxicity of grains in seleniferous areas outside of Wyoming is dependent upon the action of converter plants. They point out that twenty-five or fifty years have elapsed since dense stands of *Astragalus* or other selenium-accumulating wild plants have grown on soils that are now producing toxic wheats. They concede that danger to public health outside of the selenium area seems relatively slight. Even though toxic wheat is marketed, its dilution with non-toxic wheat and the nominal proportion of bread in the human diet tend to reduce the probable in-take to an innocuous level.

Apparently the wheat plant can tolerate a fairly high concentration of "available" selenium in the substratum or soil in which it is grown. This was emphasized by Miller and Byers³⁴ and others. On the other hand, the wheat plant will not absorb as much selenium from seleniferous soils as will plants of certain other species, which was demonstrated by these investigators, by Moxon, Olson, and Searight³¹ and others. Thus Miller and Byers found that a plant of *Astragalus bisulcatus* or two-groove poison vetch growing in a wheat field contained 1,110 pp million of selenium, while the wheat contained only 45 ppm. Incidentally, hydrogen ion concentration of the soil did not appear to exert any direct influence upon the absorption of selenium.

This tolerance of wheat to selenium in the instance of seleniferous soils may be the consequence of the presence of an abundance of sulfur compounds in the soil, for Hurd-Karrer³⁵ observed that 1 ppm of Se in soil killed wheat plants, but this toxicity was inhibited when 32 ppm of sulfur was added either in the form of sulfates or elementary sulfur. Indeed a ratio of 10 parts of S to 1 of Se appeared to inhibit toxicity of the Se even when the latter was present in fairly high concentrations.

The presence of added sulfur or gypsum, applied to a naturally seleniferous soil by Franke and Painter,³⁶ did not however, inhibit the absorption of selenium by wheat.

³³ *Botan. Rev.*, 2, 373 (1936).

³⁴ *J. Agr. Research*, 55, 59 (1937).

³⁵ *Ibid.*, 49, 843 (1934).

³⁶ *Ind. Eng. Chem.*, 29, 591 (1937).

According to Hurd-Karrer³⁷ it was suspected by Cameron³⁶ in 1880 that injury to plants caused by selenium might be due to its replacement of sulfur in some organic compound, a view which was supported later by Levine.³⁸ Hurd-Karrer³⁹ was convinced early in her investigations along these lines that the selenium present in grain is in intimate association with the protein.

Sodium selenate was much more toxic to wheat growing in Pierre clay than in Keyport clay loam in the studies of absorption of selenium from soils by wheat conducted by Hurd-Karrer.⁴⁰ In the Keyport clay loam about 15 pp million of selenium was required for distinct chlorosis, and over 30 ppm for fatal injury to the wheat plants, whereas in the Pierre clay the addition of only 3 ppm produced definite traces of chlorosis and 10 ppm was often fatal. Likewise the concentration of Se in the wheat plants grown in the Pierre clay was higher at every level of Se addition to the soil. Elemental sulfur and gypsum additions to these selenized soils reduced the absorption of selenium by the wheat, the reduction being of the order of one-half, or even two-thirds in the heavier sulfur applications.

Winter wheat grown by Hurd-Karrer and Kennedy⁴¹ on Keyport clay loam, presumably essentially selenium-free, to which 2 pp million of selenium as sodium selenate was added, when used as 70 per cent of the diet of white rats retarded the growth rate and produced the liver injury characteristic of selenium poisoning. This wheat contained about 12 ppm of selenium. When the selenized soil was treated with flowers of sulfur or with gypsum the wheat was not toxic, and the Se content of the wheat was reduced to 4 ppm.

Robinson⁴² reported the presence of 5-12 ppm of selenium in "poisonous" wheat, and 90 ppm in a gluten presumably prepared from toxic wheat.

The type of toxic wheat, later shown to owe its toxicity to the presence of selenium, was used by Franke⁴³ for the recovery of gluten, and other fractions. These were fed to test rats and from the shape of the growth curves it became apparent that the most of the toxic principle was in the protein, and not in the starch. It was further observed by Franke and Moxon⁴⁴ that when normal wheat gluten was added to a yeast-glucose mixture, the rate of fermentation was greater than when the gluten was not present. On the other hand, when an equal quantity

³⁷ *Sci. Proc. Royal Dublin Soc. (n.s.)*, 2, 231 (1880).

³⁸ *Am. J. Botany*, 12, 82 (1925).

³⁹ *Science*, 78, 124 (1933).

⁴⁰ *J. Agr. Research*, 50, 413 (1935).

⁴¹ *J. Agr. Research*, 52, 933 (1936).

⁴² *J. Assoc. Official Agr. Chem.*, 16, 423 (1933).

⁴³ *J. Nutrition*, 8, 609 (1934).

⁴⁴ *Ibid.*, 8, 625 (1934).

of gluten from a toxic wheat was added, no such acceleration of fermentation was effected. Various selenium compounds were tested by Moxon and Franke⁴⁵ to determine their effect upon yeast fermentation. It appeared that the toxicity of sodium salts of selenite, selivide, and selenate decreased in the order named. These toxic effects were not counteracted by such sulfur compounds as sodium sulfite, ammonium sulfate, or sodium thiosulfate, but sodium sulfide did conteract it considerably.

Investigations of the physiological effects of selenium compounds conducted by Franke and Potter⁴⁶ convinced them that the toxicity of selenium to test rats depends upon the ionic combination in which it is found. Thus, elemental selenium in the form of the powdered metal appeared to be virtually harmless when added to control wheat in concentrations equivalent with the amount encountered in toxic wheat. Sodium selenite, on the other hand, produced symptoms virtually identical with those produced by the natural plant toxicant. In another paper in the same series Franke⁴⁷ observed that a dilution of toxic grain (concentration of selenium not reported) to the point where it constituted 17.5 per cent of the diet resulted in depressed growth rates in young rats and even caused deaths.

After showing that the selenium is linked to the protein of toxic grains, Painter and Franke⁴⁸ hydrolyzed toxic wheat gluten with sulfuric acid and examined the hydrolysate. Marx had previously extracted such an hydrolysate with chloroform, petroleum ether, alcohol, and benzene, but no selenium was thus removed. In the present study, nearly all the selenium passed into the butyl alcohol fraction when treated with that solvent. Phosphotungstic acid as used for the precipitation of the bases actually precipitated less than half the selenium from toxic protein hydrolysates. About the same proportion appeared in a cuprous mercaptide precipitate, but only a trace was precipitated by silver sulfate in acid solution, and none by silver oxide. Mercuric chloride proved to be the best precipitant of the selenium compounds, but a saturated solution must be used. In general, such treatments with mercury salts precipitate histidine and tryptophane quantitatively, and cystine and tyrosine to a less extent, although Vickery and Cole found 86 per cent of cystine to be thus precipitated, and methionine had also been precipitated by several workers with mercury salts.

These observations served to lend support to an earlier conjecture of these authors that selenium would replace sulfur in proteins. They

⁴⁵ *Ind. Eng. Chem.*, **27**, 77 (1935).

⁴⁶ *J. Nutrition*, **10**, 213 (1935).

⁴⁷ *Ibid.*, **10**, 223 (1935).

⁴⁸ *J. Biol. Chem.*, **111**, 643 (1935).

feel that there is evidence here that most of the selenium is in a compound very similar to cystine.

On extending these studies Franke and Painter⁴⁹ found that a diet containing the sulfuric acid hydrolysate of gluten from toxic wheat, while somewhat toxic, was less so than the equivalent quantity of the protein from which it was prepared. Treatment of the hydrolysate with mercuric chloride and removal of the precipitate reduced the growth-depressing properties of the residue and left it innocuous to albino rats.

When seleniferous wheat gluten was peptized in acetic acid by Franke and Painter,⁵⁰ and then shaken with carbon disulfide, no selenium was removed. When the protein was peptized by potassium cyanide, and then dialyzed in cellophane, no selenium dialyzed out. Metallic selenium, on like treatment, was observed to dialyze readily. Even as vigorous a treatment as peptizing the protein in 0.2N NaOH and electro-dialyzing did not reduce the selenium content of the protein appreciably.

In the second paper of this series Painter and Franke⁵¹ subjected wheat protein containing about 100 ppm of selenium to (1) hydrolysis with 20 per cent HCl, and (2) hydrolysis with 33½ per cent H₂SO₄. Some selenium appeared in the humin fraction. This suggested that the selenium may have been in some compound similar to tryptophane. When the tryptophane was isolated from a seleniferous protein hydrolysate, its Se content was low, however. The same general experience was had with tyrosine.

Since selenium resembles sulfur in its chemical properties, Painter and Franke deemed it not improbable that it partially replaces the latter in the cystine and methionine portions of the seleniferous protein molecule. Even with 120 ppm of selenium in the protein used, the molar Se:S ratio is only 1:148.

Any diet containing over 5 pp million of selenium was observed by Franke and Painter⁵² to retard the growth of young rats. Diets containing more than 9 ppm caused death in young rats, but older animals exhibited a greater tolerance. With more than 10 ppm in the diet there was a pronounced reduction of food consumption. In the study conducted by Munsell, De Vancy and Kennedy⁵³ with rats, it was found that 1.5 ppm of Se in the diet was without effect upon either growth rate or reproduction, 3 ppm had a slight effect upon reproduction, although growth was normal, while with 6 ppm weight of the animals was below

⁴⁹ *J. Nutrition*, 10, 599 (1935).

⁵⁰ *Cereal Chem.*, 13, 67 (1936).

⁵¹ *Ibid.*, 13, 172 (1936).

⁵² *Cereal Chem.*, 15, 1 (1938).

⁵³ U. S. Dept. Agr. Tech. Bull., 534 (1936).

normal, and the number of young born and percentage reared was reduced.

Such solvents as water, alcohol, and ether did not remove demonstrable quantities of the toxic principle from seleniferous wheats in the experiments conducted by Horn, Nelson, and Jones.⁵⁴ The toxic principle appeared to be distributed fairly uniformly in flour, bran, and shorts milled from a toxic (seleniferous) wheat, and, moreover, was resident entirely in the protein fraction. Gliadin from toxic wheat was as toxic as the gluten.

Likewise the products of hydrolysis of gluten were as toxic as the gluten from which they were formed. In this hydrolysate the toxic properties were not associated with the dicarboxylic amino acids, or with the basic amino acids precipitated by phosphotungstic acid, but were carried by the mono-amino acids. By systematic fractional crystallization of these products of hydrolysis of wheat gluten, most of the selenium was concentrated in the amino acid fraction which was referred to as the "leucine fraction." A hydantoin was obtained on condensation as effected with potassium cyanate which was in the form of what appeared to be homogeneous crystals that contained selenium.

The wheat gluten involved in the previous study was subjected to further manipulation by Jones, Horn, and Gersdorff.⁵⁵ A papain digestate was separated into four fractions, and fraction B, which was precipitated when the digestate was brought to pH 6.2, was found to contain 58 per cent of the total selenium, and this fraction also had the highest concentration of cystine. Those fractions which contained no selenium were also lacking in cystine. Emphasis was again laid upon the observation that the dicarboxylic acids could be recovered entirely free from selenium. The study further convinced Jones *et al* that the selenium in toxic wheat is combined with the protein.

The synthesis of α , δ diamino-diselenium-dihydracryl acid (selenium cystine) was described by Fredga.⁵⁶

Selenium was separated from the protein recovered from seleniferous wheats by treatment of the protein with oxidizing agents in the experiments conducted by Westfall and Smith.⁵⁷ Bromine in hydrobromic acid and hydrogen peroxide were effective, and a quantitative removal occurred. No gross hydrolysis of the protein was evident, nor were these investigators certain as to the exact chemical constitution of the selenium-containing fraction that was removed. At least a part of the selenium separated from protein by treatment with bromine in hydrobromic acid

⁵⁴ *Cereal Chem.*, 13, 126 (1936).

⁵⁵ *Cereal Chem.*, 14, 130 (1937).

⁵⁶ *Svensk Kem. Tid.*, 48, 160 (1936).

⁵⁷ *Cereal Chem.*, 16, 231 (1939).

could be precipitated as elementary selenium by reduction with sulfur dioxide and hydroxylamine hydrochloride. So the major portion of the selenium split off by these treatments was probably inorganic, and hence this gives no clue to the chemical nature of the precursor in the protein other than that it is moderately labile.

That these observations by Westfall and Smith were in essential harmony with the assumption of organic combinations of the selenium in the protein molecule was contended by Painter and Franke.⁵⁸ They emphasized the ease with which labile sulfur-containing groups in proteins are removed by gentle alkaline hydrolysis, and demonstrated that one-quarter to one-third of the selenium can be cleaved by like treatment of gluten.

In the products of milling a sample of wheat containing 25 pp million of selenium, Painter and Franke⁵⁹ found 18 ppm in the patent flour, 30 ppm in the middlings (shorts), and 33 ppm in the bran. When gluten was recovered from flours milled from wheats containing 25 to 30 ppm of Se, the selenium content of the crude gluten ranged from 85 to 117 ppm or about four times as much. Gliadin and glutenin preparations recovered from one of these glutes contained somewhat more Se than the crude gluten, which is not surprising, since crude gluten is generally far from being pure protein material. There was some indication of a higher Se content in the glutenin than in the gliadin fraction.

Four samples of dark northern spring wheat grown in seleniferous soil in South Dakota were milled into flour, bran, and shorts, and these products were analyzed for their selenium content in a study conducted by Moxon, Olson, Whitehead, Hilmoe, and White.^{59a} The several break and reduction flour streams which were compounded into the total flour did not vary substantially in the instance of the mill streams from each individual wheat sample. They did vary directly as a function of the selenium content of the several wheats, however, and contained only slightly less selenium than the latter. Brans contained more selenium than the wheats from which they were milled, by the order of about 40 per cent, while the shorts contained less selenium than the bran, being about midway between the latter and the wheats in that particular. This is evident from the following data:

Selenium Concentration in Parts Per Million				
Sample No.	1	2	3	4
Wheat	4.8	5.8	23.3	63.0
Flour	4.1	4.1	19.1	53.6
Bran	5.9	8.7	33.4	88.4
Shorts	5.5	6.3	24.8	77.2

⁵⁸ *J. Biol. Chem.*, **134**, 557 (1940).

⁵⁹ *Am. J. Botany*, **27**, 836 (1940).

^{59a} *Cereal Chem.*, **20**, 376 (1943).

Samples of wheat from various parts of the world were examined for their selenium content by Robinson,⁶⁰ and he concluded that it is a normal constituent of the wheat grown in the great producing countries. In random selections from market wheats, the concentration was generally low, ranging from 0.1 to 1.9 parts per million. As a matter of fact, only two of over 30 samples contained in excess of 1.0 ppm and by far the majority contained less than 0.5 ppm of Se.

On the other hand, a wheat grown on a highly seleniferous soil in the northern Great Plains contained 26 ppm of Se, and wheat raised in a greenhouse on soil to which readily available Se (as sodium selenate) had been added contained 90 ppm of Se. Gluten recovered from the former contained 121 ppm of Se; from the latter, 340 ppm. This demonstrates further that the gluten of the wheat carries most of the selenium.

Saskatchewan wheats were analyzed by Thorwaldson and Johnson⁶¹ to determine their selenium content. A total of over two thousand samples was made up into 230 composites representing as many shipping points, and these composites were analyzed separately. The distribution of these composites into categories was as follows:

Selenium content pp million in group	Percentage of total composites in group
1.5	4.3
1.0	14.3
0.5	38.3
0.25	23.0
0.25	12.6
none	7.4

Of the total composites only ten, representing 4.3 per cent of the composites (and 3.2 per cent of the individual samples comprising the composites) contained more than 1.0 pp million of selenium. It is apparent that the wheats grown in the area from which these samples were collected are scarcely in the class that would be regarded as toxic (3-5 pp million or thereabouts).

Of the 951 wheat samples from the northern plains and mountain states that were examined by Lakin and Byers,⁶² 82.5 per cent contained one part per million or less of selenium, 10 per cent contained 2-3 ppm, and 7.5 per cent contained 4 ppm or more. South Dakota, Nebraska, and Kansas wheats were most prominently represented in the high selenium groups.

Of 66 samples of flour collected from mills in this area, only five contained in excess of 1 ppm of selenium. Two samples actually contained

⁶⁰ *Ind. Eng. Chem.*, **28**, 736 (1936).

⁶¹ *Can. J. Research (B)*, **18**, 138 (1940).

⁶² *Cereal Chem.*, **18**, 73 (1941).

four, and five ppm respectively, and the wheat, bran, and middlings also contained selenium in the same range of concentration.

These authors agree with Robinson ⁶⁰ that it is improbable that any field sample of wheat is entirely free from selenium, although an extra large sample may have to be used for its detection and estimation. As to what constitutes a hazardous level of selenium content, they mention rat experiments in which a diet containing 3 ppm resulted in a slight effect upon reproduction, although growth was apparently normal; with 6 ppm the animals were considerably below weight, and the number of young born and the percentage reared were less than normal.

Since flour does not commonly constitute all of the diet, and since the likelihood of mills grinding highly seleniferous wheats exclusively is remote, it does not appear from these data that there is any substantial hazard to the public from this source. Moreover, much of the highly seleniferous areas are being withdrawn from cereal production which further reduces the likelihood of the occurrence of significant concentrations of Se in American foods.

Chapter 14

Acidity

The acidic substances in cereals and flour must be discussed with less definiteness than most of the other substance present with the possible exception of the reducing materials. This is because most solvents, and particularly those employed prior to 1935, extracted a variety of compounds, and these have not been separated and identified with any degree of precision. Moreover, as will be emphasized later, when water is used as the extraction medium, it seems probable that biochemical processes go forward during the extraction period which result in the appearance of acid-reacting substances not present as such in the original flour or meal.

Apparently the method proposed by Balland in 1894, involving the extraction of flour with 85 per cent ethanol, and titration of an aliquot with turmeric (curcuma) as an indicator, was widely used in Europe. It probably constituted the basis for the so-called Greek method that occasioned so much discussion about 1929 to 1931. Since numerous variations have been made upon that general theme, summaries of twenty-five methods described in the literature have been prepared and are included in Table 92. For reasons stated in the introduction, no effort is made here to interpret the results of these acidity determinations as applied to flour or grain stability in storage, soundness, or technological properties.

Acidity of flour was considered by Balland¹ to be produced by various organic acids which are augmented upon aging the flour. Most of the increased acidity which thus results appeared to be fatty acids which are soluble in 95 per cent alcohol. Thus, the greater the fat content of the flour, the larger the increase in acidity on aging.

Acidity of flours, as determined by White's method² involving titration against phenolphthalein of an aqueous extract prepared at 40° C was found by Bailey³ to be related to the ash content of the flour. This relationship is best shown by the graph in Fig. 15. The color score of flours was also observed to decrease, *i.e.* become less white, as the acidity and the ash content of flour increased.

¹ *Compt. rend.*, 137, 724 (1903).

² *No. Dak. Agr. Expt. Sta.*, 20th Ann. Rpt. (1909).

³ "The Chemistry of Wheat Flour," p. 149, New York, 1925.

Table 92. Summary of Acidity Methods Described in the Literature.

Reported by	Details published in	Solvent	Conditions of Extractions	Indicator	Acidity expressed as
Günther	<i>Mitt. Pharm. Erlangen</i> , 2, 13 (1889).	Absolute alcohol	In Soxhlet for 12 hours	Litmus	
Balland	<i>Compt. rend.</i> , 119, 565 (1894).	85-90% alcohol	Room temp. 12 hours	Turmeric	Sulfuric
Prior	<i>Brauer. J.</i> , 4, 74 (1894).	Chloroform	14 hours		
Fachinato	<i>Gazz. chim. ital.</i> 32, 543 (1902).	85% alcohol	24 hours	Phenolphthal- ein	Sulfuric
Kreis and Arragon	<i>Schwiz. Wochenschr.</i> , 38, 64 (1900-01).	Water	Boiled 30 minutes	Phenolphthal- ein	Equivalent of N alkali
Ferreira da Silva	<i>Rev. chim. pura applicada</i> , 1, 263 (1905).	Alcohol			Sulfuric
Dombrowsky	<i>Arch. Hyg.</i> , 50, 97 (1904).	Water	Room temp.	Phenolphthal- ein	Lactic
Schindler	<i>Z. landw. Versuchsw. Deut. Oesterr.</i> , 12, 791 (1909).	85% alcohol	Room temp. 24 hours	Phenolphthal- ein	Equivalent of 0.1 N alkali
White	<i>N. D. Agr. Exp. Sta. 20th Ann. Rept.</i> , 65 (1909).	Water	40°—2 hours	Phenolphthal- ein	Lactic
Planchon	Jago and Jago, "Technology of Bread Making," p. 773, 1911.	Water	Room temp. 24 hours	Phenolphthal- ein	Sulfuric
		alcohol	24 hours	Phenolphthalein	Sulfuric
Breteau	"Guide pratique des falsifications et alterations des substances alimentaires," p. 167, Paris, 1907.	90% alcohol	Room temp. 12 hours	Litmus	Sulfuric
Girard	"Analyse des matieres alimentaires et recherche de leurs falsifications," p. 167, Paris, 1904.	85% alcohol	Room temp. 24 hours	Curcuma	
Beytheim	"Handbuch der Nahrungsmitteluntersuchung," p. 393, Leipzig, 1913.	Absolute alcohol	In Soxhlet evap. & dissolve in water	Litmus	
Rammstedt	<i>Z. angew. Chem.</i> , 26, 677 (1913).	Absolute alcohol	Boil 30 min.	Phenolphthal- ein	Equivalent of N alkali
Besley and Baston	<i>U. S. Dept. Agr. Bull.</i> , 102 (1914).	80% alcohol	16-18 hours.* Room temp.		Equivalent of 0.01 alkali

*Besley and Baston later (*U. S. Dept. Agr. Circ.*, 68, 1916) substituted high speed stirring for 30 minutes extraction.

Table 92.—Continued

Reported by	Details published in	Solvent	Conditions of Extractions	Indicator	Acidity expressed as
Lüers and Adler	<i>Z. Untersuch. Nahr. u. Genussm.</i> , 29, 281 (1915).	alcohol	Heat at 70-80° for 30 Min.		Equivalent of 0.1 N alkali
Swanson, Willard and Fitz	<i>Kans. Agr. Exp. Sta. Bull.</i> , 202 (1915).	Water Water	Room temp. 35 min. Room temp. 120 min.	Phenolphthal- ein	Lactic
Kalning	<i>Z. ges. Getreidew.</i> , 11, 105 (1919).	Acetone	In ex- traction thimble 17 hrs.	Phenol- phthal- ein	Equivalent of N alkali
Birkner	<i>J. Agr. Research</i> , 18, 33 (1919).	Cold water	Ice-bath for 1.5 hours**	Phenol- phthal- ein	Equivalent of 1.0 N alkali
Heiduschka and Deining- inger	<i>Z. Untersuch. Nahr. u. Genussm.</i> , 40, 161 (1920).	90% alcohol	20 min. on water bath, evap. & dissolve in water	Phenol- phthal- ein	
Arpin and Pecaud	<i>Ann. chim. anal.</i> , 4, 462 (1922).	90% alcohol	Room temp 24 hours	Cur- cuma	Sulfuric
Greek offi- cial method	<i>Cereal Chem.</i> , 6, 515 (1929).	85% alcohol	Room temp. 24 hours	Cur- cuma	Sulfuric
Schulerud	<i>Cereal Chem.</i> , 9, 128 (1932).	67% alcohol	25° C few minutes	Phenol- phthal- ein	cc 1.0 N alk. per 100 g flour
Association of Official Agricul- tural Chem- ists	"Official & Tentative Methods of Analysis, A.O.A.C." 5th ed., p. 363, 1940.	Benzin and ben- zin- alcohol	16 hours	Phenol- phthal- ein	mg KOH per 100 g flour
American Association of Cereal Chemists	"Cereal Laboratory Methods," 4th ed., p. 33, 1941.	Greek and Schulerud methods, as above			

** Birkner extracted corn for 1.5 hours, oats for 1 hour.

The analysis of a collection of flour mill streams produced from a single lot of wheat led Swanson⁴ to the conclusion that there was a relationship between the acidity, the amino compounds, and the phosphorus content of the samples. Reasoning from the quantities of various inorganic elements present in these flours of widely varying grade, it appeared that the soluble phosphates extracted by water were in the form of phosphates of potassium. Moreover, it appeared probable that monopotassium phosphate in such extracts might constitute a part

** Birkner extracted corn for 1.5 hours, oats for 1 hour.

although not necessarily all of the acid-reacting constituents of aqueous extracts. It was not apparent, however, as to whether such phosphates were present as such in the original flour, or appeared in the aqueous extract in consequence of hydrolysis of organic phosphorus compounds, but with the odds in favor of the latter.

Initial acidity, and rate of change on storage, was determined by Fifield and Bailey,⁵ using the Greek⁶ and the Association of Official Agricultural Chemists⁷ methods. Attention was called to the empiricism of the latter; thus, the acidity of the aqueous extract after 60 minutes digestion of flour at 40° is probably the sum of the acid-reacting materials (or, more properly, the alkali-binding and neutralizing materials) present in water-soluble form in the original flour, plus alkali-binding and neutralizing substances produced during the extraction. The conditions of extraction, in the presence of water and at a fairly warm temperature, appear conducive to enzymic action, and bacterial activity. Consequently, the determination by the A. O. A. C. method measures the capacity of the aqueous flour suspension to produce acids, as well as the initial concentration of acids before the extraction medium is applied.

A purely empirical approach to the estimation of the factor which could be used to predict acidity on the A. O. A. C. basis from acidity as determined by the Greek method was made by Fifield and Bailey. Based upon 80 comparisons or pairs of determinations the following factors appeared:

Acidity as determined by the Greek method	Multiply by this factor to convert into acidity by the A. O. A. C. method
0.05	6.0
0.10	4.2
0.15	3.3
0.20	2.4

In a continuation of the studies reported by Fifield and Bailey, a collection of 52 samples of flour representing different grades and classes was made by Markley and Bailey⁸ and subjected to collaborative study by a committee of cereal chemists. Acidity as determined by the Greek (alcohol extraction), and the A. O. A. C. method (water extraction at 40°) tended to increase progressively from the higher to the lower grade flours. The coefficient of correlation of acidity as determined by the Greek method (*g*), and ash content of the flours (*a*) was $r_{ga} = 0.60 \pm 0.06$, while a like correlation of ash and acidity by the A. O. A. C. method (*w*)

⁵ *Cereal Chem.*, **6**, 530 (1929).

⁶ *Ibid.*, **6**, 515 (1929).

⁷ "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 2nd ed., p. 225, Washington, D. C., 1925.

⁸ *Cereal Chem.*, **8**, 29 (1931).

was $r_{wa}=0.84\pm0.03$. When the acidity by the Greek method was subtracted from the acidity by the A. O. A. C. method (w-g), the correlation of this difference with ash content was almost perfect, $r_a(w-g)=0.96\pm0.01$.

When the daily increase in acidity on storage was determined by the two methods, and this increase was plotted against ash content of the flours, it became evident that the rate of change was higher as a function of ash content when the Greek method was used, than when the A. O. A. C. method was employed. The correlation between ash content and the rate of change in acidity on storage was high, and identical in the instance of both acidity methods, being $r=0.885\pm0.025$.

The correlation between acidity as determined by the two methods was $r_{gw}=0.62\pm0.06$ in the 52 flours when fresh, and after 80 days storage it increased to $r_{gw}=0.92\pm0.02$.

To control or prevent bacterial action during the extraction of acids, Schulerud⁹ added a disinfectant (dil. formaldehyde) to a flour suspension in water, and found that the increase in acidity which ensued with the lapse of time could be attributed to the increase of soluble phosphates in the extract. In alcoholic extracts no such increase occurred and he proposed the use of 67 per cent ethanol as the solvent since both the acidic phosphates, and fatty acids are soluble in it. These phosphates were not fully soluble in the 85 per cent ethanol employed as a solvent in the Greek method, however.

In an extension of this study as applied to three rye flours, and one patent wheat flour Schulerud¹⁰ found that 96 per cent ethanol extracted more acid-reacting material than did ether, by a small amount, while 67 per cent ethanol extracted decidedly more than the 96 per cent ethanol. The rate of change in apparent acidity, as determined by the use of these three solvents, on storage of a rye flour was about the same over a period of a year. A discussion of these general considerations was published by Schulerud¹¹ in another series of papers.

It was emphasized later by Zeleny and Coleman¹² that the 67 per cent ethanol recommended by Schulerud fails to determine quantitatively the amino acids extracted.

Long carbon chain acids which are soluble in ether were held by Johnson and Green¹³ as being responsible for the increase in acidity which results from the protracted storage of flour. The coefficient of distribution of these acids between water and ether was of the order 0.014 to 1.000.

⁹ *Cereal Chem.*, 9, 128 (1932).

¹⁰ *Ibid.*, 10, 128 (1933).

¹¹ *Z. ges. Getreide-Mühlen-u. Bäckereiw.*, 21, 29; 21, 68; 21, 134 (1934).

¹² *Cereal Chem.*, 15, 580 (1938).

¹³ *Cereal Chem.*, 8, 134 (1931).

The total acidity of flour extracts was observed by Melo¹⁴ to increase with age, as well as with diminishing quality. Little or no relation between acidity of extract and pH was evident. Water and ethanol as solvents did not extract the same amounts of acid from flour.

Acidic substances in flour were classified by Zeleny and Coleman¹⁵ and by Zeleny¹⁶ into three classes: (1) acid phosphates, (2) amino acids, and (3) free fatty acids. They may be increased under certain conditions by products of hydrolysis of phytin, protein, and fats respectively. The acid phosphates and amino acids are soluble in water and dilute ethanol, but are relatively insoluble in strong ethanol. Most of the amino acids are neutral in reaction in an aqueous solution, however, and accordingly would not register in the titratable acidity of a water extract of flour. In 85 per cent ethanol, the carboxyl groups of most of the common amino acids may be titrated quantitatively.

On the other hand, free fatty acids in flour are essentially insoluble in water, but are soluble in fat solvents and in strong alcohol.

To distinguish (1) "fat acidity," (2) "phosphate acidity," and (3) "amino acid acidity," Zeleny extracted flour with petroleum ether, evaporated the solvent from the extract, and dissolved the residue in benzene-ethanol (1:1) mixture, and titrated with standard alkali using phenolphthalein as an indicator. The resulting value was recorded as (1) *Fat Acidity*. The residue of flour from the petroleum ether extraction was then extracted with neutral 60 per cent ethanol, and to a portion of the extract sufficient water was added to dilute the concentration of ethanol to 5 per cent and titrated. This was termed (2) *Phosphate Acidity*. To another aliquot sufficient strong ethanol was added to bring the concentration of the latter to 85 per cent and this was titrated. The difference between this titration and the phosphate acidity represents the (3) *Amino Acid Acidity*.

When hard and soft wheat flours were stored for about 200 days, the amino acid acidity changed very little, the phosphate acidity increased slightly, but the fatty acid acidity increased three- or four-fold.

Acidity of the ether-soluble substances of flour and bran was used by Secchi¹⁷ as a criterion of refinement (ash content) and stability in storage.

A greater relative increase in the acid numbers of ether extracts of flours was observed by Arany¹⁸ in the instance of soft, white wheat flours during storage than in hard wheat flours.

¹⁴ *Rev. inst. Adolfo Lutz*, 1, 457 (1941).

¹⁵ *Cereal Chem.*, 15, 580 (1938).

¹⁶ *J. Assn. Official Agr. Chemists*, 22, 526 (1939).

¹⁷ *Ann. chim. applicata*, 30, 86 (1940).

¹⁸ *Mezőgazdasági Kutatások*, 13, 9 (1940). *Chem. Abst.*, 34, 3380 (1940). Original not seen.

Chapter 15

Wheat and Flour Pigments

Interest in the carotinoid pigments of flour grew out of the development of flour-bleaching processes. The early study of these pigments reported by Monier-Williams¹ indicated that he was convinced that the pigment extracted from flour by petroleum ether was identical with the "pure carotin" available to him. This conclusion was based upon his observations of the absorption spectra and had been supported by the contemporary studies of Wesener and Teller.²

It should be emphasized early in this discussion that much of the earlier work on flour pigments, including the researches of Ferrári and Bailey, antedated the elucidation of the constitution and classification of the carotinoids afforded by the brilliant work of Karrer, Kuhn, Zechmeister, and others. Naturally the nomenclature of these substances did not emerge until after their fractionation and recovery was effected, and much of this cataloging was done recently or is still in progress. Consequently the terms employed in the earlier work, and the techniques that were used must be interpreted or considered in the light of the knowledge of those times.

A quantitative slant was given to the study of flour pigments by Winton,³ who compared the color of gasoline extracts of flour with that of a 0.005 per cent solution of potassium chromate in water. The latter was assumed arbitrarily to have a "gasoline number" of 1.0, and on this basis eight samples of typical unbleached flours were accorded the following gasoline color values: Minnesota hard spring 78 per cent patent, 2.00; Minnesota hard spring 22 per cent clear, 2.00; Nebraska hard winter wheat 80 per cent patent, 2.63; Nebraska hard winter wheat 20 per cent clear, 2.50; Michigan soft winter wheat 80 per cent patent, 1.43; Michigan soft winter wheat 20 per cent clear, 1.61; Missouri soft winter wheat 40 per cent patent, 1.47; Missouri soft winter wheat 60 per cent clear, 1.60.

These studies of Winton's indicated that there might be a substantial range in the concentration of these yellow carotinoids in flour milled from different wheat types. They also showed, in another connection,

¹ *Reports Local Govt. Bd. (Great Brit.); Public Health and Med. Subjs.*, n.s. No. 73 (1912).

² *J. Ind. Eng. Chem.*, 3, 912 (1911).

³ *U. S. Dept. Agr., Bur. Chem. Bull.*, 137, 144 (1911).

that about two-thirds or thereabouts of the pigments were decolorized by bleaching the fresh flour with nitrogen peroxide.

The gasoline color value method was modified by Coleman and Christie,⁴ who rendered it more rapid by applying mechanical agitation in the extraction process. The extraction period was thus reduced to 20 minutes, instead of the 16-hour process described by the Association of Official Agricultural Chemists. This method was applied by them⁵ to samples of five classes of American wheats with the following results in terms of gasoline color value: durum, 1.39; hard red spring, 1.38; hard red winter, 1.68; soft red winter, 1.17; white wheat, 1.60. In a collection of 194 samples of durum wheat flour, there was no correlation evident between gasoline color value and protein content, or gasoline color value and ash content.

On applying the Coleman and Christie⁶ method for determining the gasoline color value to German wheats, Voss⁷ found that their gasoline extracts could be divided into four major groups, which he described thus:

	Subjective Color value
Strongly yellow	3
Yellow	2
Weakly yellow	1
Water clear	0

Using a photometer he measured the extinction coefficient (E) of these extracts and found them to range from $35 \cdot 10^3$ to $123 \cdot 10^3$. In the instance of individual varieties, they also varied in gasoline color value as a function of the environment in which they were grown. Thus among nine samples of Carstens Dickkopf V, of the 1935 crops, the value of E ranged from 45 to 64; Ebersbacher Weissweizen of the same crop from 56 to 80.

The recorded values for E could be converted into equivalent quantities of carotin, in parts per million by the following conversion table:

E	Calculated carotin in parts per million
35	1.02
55	1.61
75	2.19
95	2.78
115	3.36

In a further elaboration of the gasoline color value method, Jørgensen⁸ called attention to the desirability of buffering the potassium chro-

⁴ *Cereal Chem.*, 3, 84 (1926).

⁵ *Ibid.*, 3, 188 (1926).

⁶ *Cereal Chem.*, 3, 84 (1926).

⁷ *Angew. Bot.*, 18, 149 (1936).

⁸ *Cereal Chem.*, 4, 468 (1927).

mate-bichromate solutions, and indicating a preference for a solution buffered with mixed phosphates to pH=5.65, which resembles the color of a gasoline extract of flour.

Other color standards in lieu of potassium chromate (0.005 per cent) alone, in the colorimetric estimation of "gasoline color value" were proposed by several investigators. Kent-Jones and Herd⁹ added cobalt nitrate to the chromate solution to correct the latter to more nearly the hue of flour extracts. An aqueous solution of naphthol yellow and Orange G was used by Sprague¹⁰; azobenzene in 96 per cent alcohol by Kuhn and Brockmann.¹¹ Ferrari and Croze¹² found that a flour extract of known color value changed insignificantly in that property over a period of nearly 300 days when stored in a brown bottle. Of course it would be necessary to determine the actual pigment content of such a standard flour extract in advance of employing it as a reference standard in colorimetry.

The examination of flour extracts was refined and rendered more nearly quantitative by Ferrari and Bailey¹³ who first applied the methods of spectrophotometry to such determinations. They prepared a crystalline "carotin" from carrots and determined its specific transmissive index. The value, as observed using monochromatic light of a wave length of 435.8 m μ was 1.9165, which was practically identical with the average value of 1.9148 previously reported by Schertz.¹⁴ Substituting this value in a suitable equation, it was possible to convert the observed transmittancy of a gasoline extract of flour into equivalents of "carotin" concentration. From this, in turn, could be computed the parts per million of the pigment in the original substance under examination. The method was applied by Ferrari and Bailey¹⁵ to a large number of flour samples, representing various hard spring wheat varieties, about two-thirds of which were observed to contain between 2 and 3 pp million of "carotin."

In a collection of flour mill streams, the refined primary middlings flours contained an average of 2.40 ppm, the break flours (excluding the 1st break) 2.70 ppm and the secondary middlings and tailings flours an average of 2.60 ppm. The wheats from which these flours were milled contained 3.40 ppm, and the by-products as follows: bran, 2.28; shorts, 3.40; red dog, 2.85 ppm.

Changes in carotinoid concentration on storage of natural flour under

⁹ *Analyst*, 52, 443 (1927).

¹⁰ *Science*, 67, 167 (1928).

¹¹ *Z. physiol. Chem.*, 206, 41 (1932).

¹² *Cereal Chem.*, 11, 511 (1934).

¹³ *Cereal Chem.*, 6, 218 (1929).

¹⁴ *J. Agr. Research*, 26, 383 (1923).

¹⁵ *Cereal Chem.*, 6, 847 (1929).

different conditions, effect of bleaching agents, and other factors were also studied at some length by Ferrari and Bailey.¹⁶

Some further refinements, both in the instruments, including light sources, and in the extraction methods were described by Ferrari.¹⁷ He proposed the use of a mixed solvent for the carotinoids composed of light cleaner's naphtha, 93 parts, and absolute alcohol, 7 parts by volume. This solvent effected the extraction of more of the carotinoid pigments than did petroleum ether, or high test gasoline alone. It was proposed later by Whiteside, Binnington and Geddes¹⁸ that the proportion of absolute alcohol be increased to 10 per cent in order to effect maximum extraction of the carotinoids, possibly in consequence of a difference in the composition of the cleaner's naphtha available to them.

A critical study of several methods for estimating the carotinoids of flour was conducted by Geddes, Binnington and Whiteside.¹⁹ When a mercury-vapor arc was used as the source of light in colorimetric measurements of gasoline color values, the latter were found to be closely correlated with spectrophotometric measurements. The coefficient of correlation between the two sets of observations in the instance of a series of 358 flours of varying pigment concentration was $r=0.985$. The equation for computing "carotene" concentration (c) from gasoline color value (G) became: $c=-0.017+2.2571G$. The experimental error of measurements with the Duboscq colorimeter, as used in measurements of G , was significantly greater than for the spectrophotometric measurements (c), although these authors suggest that the G values may be adequate for technical requirements, and can be had at much lower cost for equipment.

When the percentage of absolute alcohol in the mixture with naphtha was progressively increased, the quantity of carotinoids extracted from flour increased rapidly to a concentration of 3 per cent of alcohol, remained constant to 10 per cent of alcohol, and then fell off sharply when the alcohol content was increased to 11 per cent, in the studies conducted by Whiteside, Binnington, and Geddes.²⁰ With wheat, however, the apparent carotinoid pigment concentration increased with increasing ethanol content of the mixed solvent until a minimum concentration of 8 per cent of ethanol was reached, and remained constant thereafter until the ethanol concentration was at least 25 per cent.

In a series of 7 flours which were arranged by these researchers in order of increasing carotinoids concentration, as determined by extrac-

¹⁶ *Ibid.*, 6, 457 (1929).

¹⁷ *Cereal Chem.*, 10, 277 (1933).

¹⁸ *Proc. World Grain Exhibition & Conf. (Regina, Can.)*, II, 438 (1933).

¹⁹ *Cereal Chem.*, 11, 1 (1934).

²⁰ *Proc. World's Grain Exhibition and Conference*, II, 438 (1933).

tion with naphtha, the quantity of pigment extracted with a mixed naphtha-alcohol solvent (93:7) also increased, but not to a uniform degree. In fact, there were some indications of a trend in the direction of a diminishing difference of the findings by the two methods as the carotinoid pigment content increased, but here again there was no definite regularity. The average difference in terms of percentage increase of the naphtha-alcohol mixture over naphtha alone was about 30 per cent of apparent carotinoid extracted. With two extended series of wheat samples representing a high and a low carotinoid variety, the average increases on the inclusion of ethanol in the solvent were 50 and 64 per cent, respectively.

With whole wheat, bran and shorts the differences were even greater, and in the order named, reaching a ratio of 2.0:1 or more with the feeds, where with the flour it was 1.3:1. With germ the ratio was 2.4:1 in one comparison. Ammoniacal alcohol-naphtha mixture extracted more soluble pigments (calculated as carotene) from wheat than did naphtha alone, the ratio being 1.2:1, and 1.35:1 in two sets of comparisons.

High carotinoid values appeared to be associated with lack of plumpness in the wheat samples. In a series of Marquis wheat samples the correlation was $r = -0.512 \pm 0.145$, and in series of Garnet samples $r = -0.758 \pm 0.077$.

Carotinoids in whole wheat were determined in several series of samples by Goulden, Geddes and Whiteside,²¹ using two different methods of extraction: (N) solvent naphtha, and (a) solvent naphtha plus alcohol (90:10). In one collection involving thirty H-44-24 x Reward hybrid spring wheats grown at Winnipeg in 1933 the averages were: (N) 1.53 ppm of carotinoids (calculated as carotene); (a) 2.61 ppm. In the flours from these wheats, the carotinoids extracted with naphtha-alcohol (93.7) mixed solvent (f) was 2.05. In another series of forty-eight H-44-24 x Marquis spring wheat hybrids grown at Winnipeg in 1933 the corresponding average carotinoid values were: (N) 1.31; (a) 2.53; (f) 2.01. With a miscellaneous group of varieties and hybrids: (N) 1.39; (a) 2.53; (f) 2.21.

It thus appears that the mixed solvent extracted considerably more yellow pigments from the wheat than did naphtha alone, and by the order of 71-93 per cent. When mixed solvents were employed in extracting the yellow pigments from wheat (a) and the flour milled therefrom (f), the values for the wheat (a) were substantially higher, by the order of 14-27 per cent.

The relationships between these several variables is further indicated

²¹ *Cereal Chem.*, 11, 557 (1934). Also private communication giving the averages quoted here (Goulden: June 25, 1941).

by the correlation coefficients as computed from these data. The correlation between the two methods of extracting whole wheat was $r_{Na}=0.7225$, and that between wheat and flour carotinoids as extracted with mixed solvents $r_{fa}=0.6584$.

No significant or appreciable correlation was found between carotinoid content of wheat and the plumpness of the kernels as indicated by either weight per average kernel or weight per bushel. Nor was there any correlation between the carotinoids in the flour and protein content of the latter in the instance of the miscellaneous group of strains and varieties of wheat, but in the series of H-44-24 x Marquis hybrids this correlation attained the significant value $r_{fp}=0.4692$.

Many pure solvents were tested by Binnington, Sibbitt and Geddes²² in extracting carotinoids from flour, including hydrocarbons and their halogen derivatives, alcohols, an aldehyde, ethers and oxides, esters, ketones and furans. With a single flour sample the apparent carotene equivalent as determined through the use of these solvents ranged from 0.17 ppm for *n*-butylaldehyde to 2.62 ppm in the instance of octyl alcohol. The alcohols in general appeared to occupy a favorable position to the extent that the amount of apparent pigment extracted was greatest when they were used as solvents. Methyl amyl (secondary) alcohol, and *n*-butyl alcohol saturated with water (19.5 per cent by weight) appeared to offer the greatest advantages, and of these the former was highest in price and lowest in purity.

Accordingly, the specific transmissive index of carotene, prepared by these investigators from carrots, and of xanthophyll, recrystallized from a commercial product, were determined in *n*-butyl alcohol as solvent and found to be 1.6632, and 1.7225, respectively. In ethyl ether the xanthophyll gave a value of 2.094, and the carotene in petroleum ether 1.9145. The value for the latter reported earlier by Schertz²³ was 1.9148, and by Ferrari and Bailey²⁴ 1.9165; for xanthophyll in ethyl ether Schertz²⁵ reported 2.089. The transmittancy measurements in these instances were made with monochromatic light, wavelength 435.8 μ .

Binnington, Sibbitt and Geddes²² employed the specific transmissive index for their carotene preparation in *n*-butyl alcohol in constructing a conversion chart which would convert the observation in transmittancy into terms of carotene in ppm. It was recognized, however, that this becomes a somewhat arbitrary calculation, since xanthophyll and other pigments had previously been shown to constitute the bulk of the flour pigments. To be sure, the same general error had long been attached to

²² *Cereal Chem.*, 15, 119 (1938).

²³ *J. Agr. Research*, 26, 888 (1923).

²⁴ *Cereal Chem.*, 6, 218 (1929).

²⁵ *J. Agr. Research*, 30, 263 (1925).

the estimation of flour pigments extracted by the mixed naphtha-alcohol solvent.

A series of 150 flours was then subjected to the determination of a carotinoid content using both naphtha-alcohol, and water-saturated *n*-butyl alcohol as solvents. The average value with the former was 1.77 ppm (as carotene), and with the water-saturated *n*-butyl alcohol 2.33 ppm. The correlation between the two sets of determinations was high, $r=0.9325$, and the regression equation became:

$$\text{carotene (butyl-alc.)} = 0.14 + 1.2377 \text{ carotene (naphtha-alc.)}$$

Stress was laid upon the advantages of a water-saturated solvent, such as these *n*-butanol preparations, over naphtha-alcohol in working with flours of high moisture content. They suggest that naphtha-alcohol may actually separate into two phases under those conditions, although an American laboratory which has used such a reagent extensively reports that such a separation never occurs.

Spectral distribution curves of extracts of high-grade and low-grade flours with both naphtha-alcohol and water-saturated *n*-butanol were presented. The same absorption bands appeared in the instance of both solvents. In progressing from the high- to the low-grade flour there was little change in the actual level of these absorption bands, in terms of transmittancy when *n*-butanol was the solvent. When naphtha-alcohol was used, however, the level of these bands tended to flatten out in passing from the high- to the low-grade flour, which suggested the presence of more flavones. This assumption received further support from the observed indicator effect of added alkali. Thus the reduction in transmittancy effected by adding ammonia to the two extracts of high-grade flour was equivalent to 0.44 ppm of carotene with the naphtha-alcohol solvent, and 0.49 ppm with water-saturated *n*-butanol. With the low-grade flour the corresponding values were 0.65, and 0.38, respectively. It is generally agreed that low-grade flours contain much more flavone; and the greater decrease in transmittancy observed on rendering the naphtha-alcohol extract alkaline with ammonia also indicates the possible presence of more flavones in low-grade flour.

The use of *n*-butanol as a solvent was fitted into a rapid method for the determination of wheat and flour pigments by Binnington and Geddes.²⁶ On extending the study of its application collaboratively Binnington²⁷ evolved the regression equation for expressing the relation of carotinoids determined by the two methods which became:

$$\text{Carotinoids (butanol)} = 0.21 + 1.219 \text{ carotinoids (naphtha-alcohol).}$$

²⁶ *Cereal Chem.*, 16, 252 (1939).

²⁷ *Ibid.*, 17, 639 (1940).

As the knowledge of the carotinoids unfolded during the period from 1930 to 1940, and new facts and methods became available, studies of the flour pigments were re-opened. There was some basis for the assumption that the principal pigment of the wheat grain was not necessarily carotene. Coward²⁸ and Hanna,²⁹ using different methods, had established the likelihood of the presence of substantial proportions of xanthophylls in wheat leaves. Even earlier Palmer³⁰ had found wheat bran to contain xanthophyll. Bowden and Moore³¹ isolated what appeared to be a xanthophyll from wheat germ oil, and Gisvold³² obtained crystalline xanthophyll from that source.

About this time doubt began to be cast upon the adequacy of the earlier observations of absorption spectra as a means of sharply identifying the individual carotinoid pigments, either alone or in mixture, without supplementary evidence. Accordingly, Markley and Bailey³³ applied a modification of the Willstätter and Stoll³⁴ method to the solvent fractionation scheme. When naphtha extracts of flour were shaken with 90 per cent methanol, the separation was vague and indefinite. If the naphtha extract was shaken with alkaline aqueous methanol, the separation was sharp and clear. Two saponifications with 11 per cent KOH in 85 per cent methanol for 30 minutes each in a shaking machine, followed by two extractions with 90 per cent methanol, appeared to remove the major part of the xanthophylls. Carotene, and lycopene, if present, could be expected to remain in the naphtha layer so treated.

These two major solvent fractions were then studied with the spectrophotometer, and two absorption bands were observed in each, but with the bands shifted to the right, *i.e.*, in the direction of longer wave length, in the naphtha-soluble pigment fraction. Moreover, the characteristics of the spectral analysis of the latter did not suggest the presence of appreciable proportions of lycopene.

This method of fractionation was given a quantitative slant by determining the proportion of the carotenes which remained in the naphtha fraction after saponification and washing out the xanthophylls. In the instance of the highly pigmented Marquillo wheat, 35 per cent of the original carotinoid concentration appeared in the residual naphtha layer after the xanthophylls and other methanol-solubles had been removed. With Marquis wheat, which is more lightly pigmented, only 13 per cent

²⁸ *Biochem. J.*, 18, 1114 (1924).

²⁹ *Can. J. Research*, 4, 134 (1931).

³⁰ "Carotinoids and Related Pigments," Chemical Catalog Co. (Reinhold Publishing Corp.), New York, 1922.

³¹ *Nature*, 132, 204 (1933).

³² *J. Am. Pharm. Assn.*, 29, 812 (1940).

³³ *Cereal Chem.*, 12, 33 (1935).

³⁴ "Untersuchungen über Chlorophyll", Julius Springer, Berlin, 1913.

remained in the same fraction. Extracts of two Mindum (durum) wheat samples occupied an intermediate position, with 17 and 22 per cent respectively in the non-methanol-soluble fraction.

When the xanthophylls were removed from the methanol washings, a chocolate-brown residue remained. A portion of this could be dissolved in carbon disulfide, yielding an orange-red solution. The exact nature of this pigment was not disclosed.

After the removal of the xanthophylls from the original crude xanthophyll extracts in methanol, a fraction containing a pigment apparently identical with Anderson's^{53,54} *tricin* was obtained in solution in ether. Other flavones, of undetermined characteristics may also have been present in the non-xanthophyll complex.

Separation of carotene and xanthophyll fractions by differential solubility after cold saponification did not prove wholly satisfactory to Munsey³⁵ when applied directly to naphtha-alcohol extracts of flours. About 50 samples of flour were thus analyzed, with results ranging from 5 per cent of the total color as carotene in durum flour, to 54 per cent in bread flours. Inconsistent results were obtained upon re-analysis at a later date.

Recourse was then had to vigorous saponification by heating on the steam bath with ethyl alcohol saturated with KOH. This reduced the apparent proportion of carotene to a much lower level, with a maximum equivalent to 0.2 ppm, and with the majority of the 71 samples ranging around 0.1 ppm. Moreover, a comparative saponification treatment applied to a specimen of pure carotene did not result in any appreciable destruction of the latter. Accordingly, Munsey concluded that practically all the carotinoid pigment of flour is xanthophyll.

Xanthophyll was separated from a wheat-germ oil by Bowden and Moore.³⁶ It was crystallized from petroleum ether, and its absorption spectrum was compared with a specimen of xanthophyll supplied by Karrer. The curves were practically identical, with heavy absorption in the visible with three maxima at 477, 444, and 418 m μ , and a weaker absorption in the ultraviolet with two maxima at 337, and 271 m μ .

Commercial wheat germ that had been extracted with trichloroethylene by Drummond, Singer and MacWalter³⁷ was found to contain lutein, and kryptoxanthin as well as anyrin and squalene. The lutein was adsorbed from a light petroleum solution, near the top of a column of "aluminum oxydatum anhydricum, nach Brockmann" Merck where it was strongly adsorbed in a deep brownish-yellow layer. The krypto-

⁵³ *J. Assoc. Official Agr. Chem.*, 21, 331 (1938).

⁵⁴ *Nature*, 132, 204 (1933).

³⁷ *Biochem. J.*, 29, 456 (1935).

xanthin was less strongly adsorbed and eventually washed out of the column into the receiving flask.

A spectrographic study of wheat grains by Euler and Malmberg³⁸ showed the presence of xanthophylls, and only a trace of carotene. In wheat embryo they estimated 5 μg of carotinoid per g, containing only 0.05 μg (or 1 per cent) of carotene.

Further emphasis upon the prominence of xanthophylls among the hydrocarbon-soluble pigments of flour was laid by the chromatographic adsorption studies of Houk.³⁹ The adsorption column was packed with active magnesium oxide obtained from the same source and, presumably, the same batch as the magnesium oxide used by Dr. H. H. Strain at Stanford University. The MgO was mixed with an equal weight of a siliceous earth to obviate too dense packing of the absorbent. The flour pigments were dissolved in petroleum ether at the time of passing the solution through the adsorbent column, and in these particular studies the pigments were unsaponified.

No less than four adsorption zones or layers could be discerned in the chromatograms in addition to the dissolved pigment which passed through. These several layers or zones were separated mechanically, and the pigment or pigments in each was eluted by a hexane-alcohol (95:5) mixture. Spectrographic analysis was applied to each of these four preparations and there was evidence of some differences. The upper zone pigment yielded spectrograms resembling the violaxanthin type, while zones 2, 3 and 4 were somewhat similar and of the lutein type, although zones 3 and 4 differed somewhat in that the band at 472 $\text{m}\mu$ was less prominent, relatively. Incidentally, it was band 4 that tended to disappear most promptly when benzoyl peroxide was applied to flour as a bleaching agent.

Most of the pigment of the extract of natural or unbleached flour in petroleum ether appeared in one or the other of these several bands. This was interpreted to imply that the bulk of the pigment present was of the xanthophyll, or xanthophyll ester type.

Zechmeister and Chohnoky⁴⁰ found that unbleached wheat flour (So. Hungary) contained (if any) no more than 0.01 (=0.01 ppm) mg of carotene per kg, and hence had no provitamin A. Practically the only polyene found was a xanthophyll, which was crystallized and identified as lutein, 15 mg of which was recovered from 60 kg of flour. Xanthophylls recovered from chromatograms varied between 1.0 and 2.5 mg per kg of flour (= 1. to 2.5 ppm).

³⁸ *Arkiv Kemi, Mineral. Geol.*, 12B, No. 14 (1936).

³⁹ Thesis, "The fractionation of wheat carotinoids," filed in the Library of the University of Minnesota, 1938.

⁴⁰ *J. Biol. Chem.*, 135, 31 (1940).

In a similar study of American whole-wheat flour Zechmeister and Escue⁴¹ recovered 1.5-2.0 mg/kg of total pigment from the flour, and on chromatographic fractionation this included 0.01-0.04 mg/kg of carotene and 1.0-1.4 mg/kg of xanthophylls. Thus the carotene fraction represents only 1-2 per cent of the total pigment. Cryptoxanthin was not present.

Canadian wheat varieties were subjected to a detailed study of their "carotin" concentration by Whiteside,⁴² who extracted the pigments with gasoline, but expressed the results in ppm of "carotin." When the same varieties were grown on dry land and on irrigated fields, the carotinoid content of the former was invariably higher, although the differences were not constant, but ranged from 0.11 to 1.19 ppm. The average for 22 samples of hard spring wheat grown on dry land and irrigated land were 2.24 and 1.74 ppm respectively, and the coefficient of correlation between these two variables was $+0.7238 \pm 0.0685$.

In the instance of the dry-land series of spring wheats there was a negative correlation between test weight per bushel and carotinoid content, $r = -0.6663 \pm 0.0800$, but no correlation was found between these characteristics in the instance of the same varieties grown on irrigated land. The same general relationships were encountered on comparing weight per thousand kernels and carotinoid content. With the dry-land series, the correlation between these variables was -0.5802 ± 0.0604 , while with the irrigated series $r = 0.0561 \pm 0.1434$, which is not significant and in fact suggests no correlation.

Fineness of grinding of wheat was found to have a decided bearing upon the facility with which carotinoids were extracted when gasoline was applied once to the ground material. When a certain wheat sample was ground on the Wiley mill to pass a 2-mm mesh, only 1.10 ppm of total carotinoids was extracted from it. With the same mill, provided with a 0.5-mm sieve, 1.52 ppm was extracted, while the same wheat ground very fine through a Seck mill yielded 2.17 ppm. Pre-digestion of the ground material with ammoniacal-alcohol appeared to facilitate extraction of the carotinoids.

A fairly high correlation between carotinoid pigments in wheat and in flour of the order of $r = 0.7$ was encountered in a collection of 139 samples examined by Goulden, Geddes and Whiteside.⁴³ This relationship was sufficient to justify the application of carotinoid determinations to wheat samples in plant breeding work.

Carotinoid concentration in wheat, while an inherent characteristic,

⁴¹ *Proc. Natl. Acad. Sci.*, **27**, 528 (1941).

⁴² *Dept. Agr. (Canada) Bull.* 154, new series, 1931.

⁴³ *Cereal Chem.*, **11**, 557 (1934).

may vary considerably as a function of the environmental conditions under which it is grown, as demonstrated by the observations of White-side, Edgar, and Goulden.⁴⁴ In general, those environmental conditions which tended to produce lower bushel weight and lower kernel weight tended also to produce higher carotinoid values. Also it appeared that the carotinoid content of flour produced from plump wheat will be lower than when the reverse is true.

Completeness of extraction of carotinoids was facilitated by fine-grinding in the experiments of Worzella and Cutler.⁴⁵ Among 28 soft and semi-hard wheat varieties grown at LaFayette, Indiana in 1933, the range in "carotene" in ppm was from 1.75 to 3.80, using naphtha-alcohol as the solvent. Of these 28 varieties, 17 had a "carotene" content between 2.00 and 2.99 ppm, and the average was 2.31 ppm. The three varieties that were highest in carotinoids, namely Progeny No. 2 (Ill.), Minturki, and Minhardi, and containing 3.80, 3.69 and 3.54 (1933 crop) ppm are closely related to the hard wheats.

The association between the carotinoid content of 72 hybrid strains of the 1932-33, and the 1933-34 crops was high, with the coefficient of correlation $r=0.904$. Likewise the interannual correlation coefficient in the instance of 27 varieties was $r=0.960$, emphasizing that carotinoid content is an inherited varietal characteristic.

Carotinoid pigment content of Federation wheat grown in the western states was found by Fifield *et al.*⁴⁶ to be higher than five other common varieties. The average of these six varieties was 2.28 ppm, while the first patent flour milled from them contained 1.34 ppm, and the second patent 1.75 ppm stated as "carotene." Naphtha-alcohol (93.7) mixed solvent was used in extracting the pigments.

In another series of samples these investigators found the following concentrations of carotene in ppm in mill products (average of six Pacific northwest wheats: Wheat, 2.02; first patent, 1.84; second patent, 1.99; first clear, 1.57; middlings, 2.95; bran, 3.33).

Pieri⁴⁷ was of the opinion that the older (Italian) wheat varieties contained more carotene than recent varieties obtained by hybridization.

Carotinoid pigment content of 21 *durum* wheat samples was found by Binnington and Geddes⁴⁸ to average 3.28 ppm while the semolinas milled from these wheats averaged 2.87 ppm. The American Association of Cereal Chemists method for carotinoid pigment determination, involving mixed naphtha-alcohol solvent, was used in these studies. Binnington

⁴⁴ *Cereal Chem.*, 11, 615 (1934).

⁴⁵ *Cereal Chem.*, 12, 708 (1935).

⁴⁶ *Cereal Chem.*, 13, 463 (1936).

⁴⁷ *Atti soc. ital. progresso sci.*, 23, III, 242 (1935); *Chem. Abs.*, 29, 6921 (1935). Original not seen.

⁴⁸ *Cereal Chem.*, 14, 263 (1937).

and Geddes also found that with one group of 13 *durum* wheats of the 1934 crop, the carotinoid pigment content averaged 2.56 ppm in November, 1934, and this had decreased to an average of 2.19 ppm on storage until August, 1935. Semolinas milled at the latter date averaged 1.76 ppm of carotinoids.

A coefficient of correlation of -0.72 was found by Markley⁴⁹ between kernel weight and carotinoid concentration in a collection of kernels harvested from 72 plants of Minn. No. 2303 (later named *Thatcher*) wheats. When the carotinoids were recorded as weight of pigment per kernel, the correlation became positive and $r=0.55$. In two lots of H-44 wheat it was greater, $r=0.84$ and 0.71 , respectively.

Using water-saturated butanol as the solvent, Geddes⁵⁰ reported the following data for his studies of three classes of wheat, and flours milled from them:

1939 Crop Class	No. of Samples	Carotinoids in ppm Mean	Standard Deviation
Hard red spring wheat	121	5.65	1.01
Hard red spring flour	121	2.95	0.44
Hard red winter wheat	45	5.81	0.62
Hard red winter flour	45	4.11	0.55
<i>Durum</i> wheat	89	7.27	1.40

It is evident that there is much more difference between the flours milled from the hard spring and hard winter wheats than between the wheats themselves, so far as the carotinoid pigment content is concerned. The hard winter wheat flours contained an average of over 50 per cent more of the butanol-soluble pigments. This suggests that the distribution of the pigments in the kernels must be quite different in these two wheat classes. In the instance of the spring wheats the coefficient of correlation of the wheat, and the flour carotinoids was $r=0.619$, and the regression equation was: Flour pigment (ppm) = 1.518×0.254 wheat pigment (ppm).

For the hard red winter wheats the corresponding statistical constants were:

$$r=0.450$$

$$\text{Flour pigment} = 1.814 \times 0.396 \text{ wheat pigment}$$

The *durum* wheats contained the highest content of butanol-soluble pigments of the three wheat classes.

As early as 1906 Simpson⁵¹ noticed that flour gave a more or less strong color with alkalis, and in 1926 he observed that with high-grade flours this color was a weak yellow, which was stronger with low-grade

⁴⁹ *Ibid.*, 14, 400 (1937).

⁵⁰ Unpublished data, 1941.

⁵¹ *Cereal Chem.*, 12, 569 (1935).

flours, intense with germ, and that the brown color of bran from red wheats became a strong orange-brown, while bran from white wheats turned an intense yellow. This indicated to him that the flavones were responsible for this behavior with alkalis and that they were more strongly concentrated in the envelope of the wheat kernel than in the endosperm.

Simpson further observed that the carotinoids could be almost completely extracted from flour by cold (100 per cent) acetone, and that flavones could then be extracted from the residue with 80 per cent acetone. When ammonia was added to the latter, a clear yellow color resulted which could be measured with a colorimeter against a 0.01 per cent aqueous potassium chromate solution (pH 6.2).

In the instance of five flours examined by him the "carotene color" values were 13.15, 15.5, 9.5, 19.25 and 11.62, and the "flavone color" values were 8.0, 8.75, 14.62, 13.37, and 17.62, respectively. Evidently there was no correlation between the two sets of color values. He then indicated the relationship between flour grade, ash content and "flavone color" recorded in Table 93.

Table 93. Ash and Flavone Content of Flours of Different Grades, as Reported by Simpson ⁵¹

Grade	Ash (%)	Flavone color
Top patents	0.35	9
1/- Patents	0.42	14
Standard flour	0.45	16
Households flour	0.50	20
Specials flour	0.55	24
Bran	6.00	600
Germ	4.50	1600

Simpson emphasized the usefulness of "flavone color" as an index of flour grade in the instance of self-rising flours, or flours to which mineral improvers have been added, and where ash determinations cannot be directly applied.

The reddish-brown pigment present in the bran of "red" wheats was presumed by Kent-Jones and Herd ⁵² to be extracted by shaking flour or meal with an alkaline methanol (about 65 per cent) solution. Glutenin was precipitated from the extract by adding HCl to pH 6.4, and alkali was again added to an aliquot of the filtrate. This was examined in a colorimeter, using a mixed potassium chromate, and cobalt nitrate solution as the color standard.

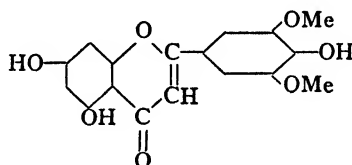
Data from two series of flour are presented, including a set of mill

⁵² *Analyst*, 52, 443 (1927).

streams. A few samples are selected from the latter as fairly typical of the trend of the relationship between ash content and the "methyl alcohol figure."

Flour mark	Color value of alkaline methyl alcohol extract	Ash (%)
B	5.0	0.30
D	6.0	0.36
F	10.0	0.53
J	19.0	0.88
M	21.0	0.93

Tricin, a trihydroxy dimethoxy-flavone, was extracted by Anderson ⁵³ from the leaves of young Khapli wheat plants, using 95 per cent alcohol as the primary solvent. The crude tricin that was first recovered represented about 260 ppm of the dried tissues, and when purified, about half of that proportion was obtained as pale yellow needles. Marquis wheat on like treatment yielded about 5 per cent of that amount of crystalline material which was identified as *o*-triacetyl tricin. Anderson ⁵⁴ then isolated about a gram of fairly pure tricin from Khapli wheat leaves, and, upon a study of its physical properties and chemical reactions, concluded that it contained the syringic nucleus, and was therefore tricetin 3', 5'-dimethyl ether, represented by the formula:



These two varieties also contained water-soluble tinctorial substances which appeared to be glucosides of tricetin or some closely related compound.

Alcohol was observed by Markley and Bailey ⁵⁵ to be an excellent solvent for pigments of the flavone type present in wheat. When 85 per cent ethanol was used, the depth of yellow color in the extract was apparently a function of the grade of flour; 50 to 67 per cent ethanol could also be employed. The hue of the extract was yellow with a slight brownish tint, as distinguished from the orange-yellow of carotene or the lemon-yellow of xanthophylls. On rendering the alcoholic solution alkaline (it is originally somewhat acid) it becomes greenish-yellow in color, and on storage for several days the brownish cast becomes pro-

⁵³ *Can. J. Research*, 7, 285 (1932).

⁵⁴ *Ibid.*, 9, 80 (1933).

⁵⁵ *Cereal Chem.*, 12, 40 (1935).

gressively more apparent. On shaking the original alcoholic extract with naphtha very little pigment appears in that solvent, which suggests the presence of little carotinoid pigment in the alcoholic extract.

Aqueous acetone also extracted pigments similar to those in the alcoholic extract. On determining the transmittancy of 50 per cent aqueous acetone extract of *Mindum durum* wheat with a spectrophotometer, a heavy absorption band was discerned at 435.8 $m\mu$, with a minor band at 525 $m\mu$ and possibly one at 565 $m\mu$. There was little extinction above 590 $m\mu$. This spectral absorption curve had characteristics in common with those previously observed by Ferrari and Bailey⁵⁶ in the gasoline extracts of wheat bran.

These observations were then extended into the ultraviolet, using a quartz spectrograph, and with the pigment dissolved in 67 per cent ethanol to avoid the pronounced absorption spectrum of acetone at low wave lengths. Also a more dilute solution was necessary because of the high extinction coefficients encountered. At a wave length of 237 $m\mu$ the absorption of light was rising almost vertically. A strong absorption band was evident at 269 $m\mu$, and a weaker one at 325 $m\mu$. From this point the extinction coefficient approached zero as the wave length increased toward 400 $m\mu$. Whether only one substance was responsible for the characteristics of the absorption curve was not fully apparent.

The preparation was then made alkaline and the extinction coefficients re-determined. The absorption bands at 269 and at 325 $m\mu$ were not shifted, but the bands were more intense. This is in line with the apparent increase in pigment concentration in the visible range of the spectrum when such solutions are rendered alkaline.

Since there is the possibility of condensation or polymerization reactions in alkaline acetone extraction processes, it is not certain that such treatments reveal the true red pigment concentration in the bran tissues of wheat.

The gasoline extract of washed bran (i.e., bran free from flour or endosperm) had an absorption spectrum with no similarity to that of either carotin or flour extract. The extract was highly pigmented, however, but its color was essentially reddish-brown.

After observing that when extracts of flour, using dilute alcohol as the solvent, were rendered alkaline, the color intensity increased decidedly, Schulerud⁵⁷ evolved a quantitative method for estimating the degree of color increase. This involved the development of the color by the addition of alkali to one aliquot; the addition of a standard color solution containing Auramin and Eosin to another aliquot. The quantity

⁵⁶ *Ibid.*, 6, 218 (1929).

⁵⁷ *Mühlenlab.*, 3, 13 (1933).

of this color standard required to effect a match between the original or slightly acid extract, and the extract after it was rendered alkaline, was termed the "Color Number" ("Farbzahl"). The latter value ranged between 0.55, in the instance of a flour containing 0.36 per cent ash, and 7.1 in a hard spring wheat second clear containing 1.23 per cent ash. In a collection of flours involving a considerable number of samples there appeared to be a fairly high correlation between ash content and the color number, as judged from the graphic array of the corresponding values. Two wheat samples gave color values of 11.4 and 12.2 respectively, and wheat bran gave the high value of 65.

This method was then refined by Schulerud⁵⁸ to include the determination of the extinction coefficient of the alkaline extracts with a photometer. It appeared that there was a linear relation between the extinction coefficient, and the ash content in a collection of about 33 flour samples of widely varying grade.

Schulerud did not accept the conclusion reached by Kuhl⁵⁹ that the coloration with alkali resulted from reactions of starch split-products. He held that the pigment involved originated in the branny envelope, and that it exhibited a true indicator effect when the pH of the extract was increased progressively from pH 7 to 9, at which level the maximum yellowness was reached. Schulerud was apparently prepared to assume that it was a flavone, such as had been described by Anderson.⁶⁰

"Indicator number" was the term later applied by Schulerud⁶¹ to the color characteristics of extracts of cereal grains. Dilute HCl was used as the extraction medium, being heated with the finely divided meal for 45 minutes in a boiling water bath. After cooling, an excess of alkali was added, back-titrated with dilute HCl to neutrality, as indicated by the color changes of the natural pigment as indicator, diluted with alcohol to a concentration of about 40 per cent of the latter, and two drops of strong alkali solution was added to render it definitely alkaline. The slight turbidity was removed by double filtration. The degree of yellow coloration was then measured with a photometer. "Indicator number" was arbitrarily defined as the extinction coefficient of the extract of 10 g of cereal made to 100 cc, observed in a layer 1 cm thick through spectral filter S43 in a Pulfrich photometer.

The test was applied to several local varieties, each of which exhibited some variability as a function of the environment in which it was grown.

The present author doubts that the coloration effected by the addition of alkali to an aqueous, or an acidified aqueous extract of wheat is due

⁵⁸ *Ibid.*, 3, 137 (1933).

⁵⁹ *Mühlenlab.*, 3, 35 (1933).

⁶⁰ *Can. J. Research*, 7, 285 (1932).

⁶¹ *Mühlenlab.*, 6, 129 (1936).

to a "pigment" exhibiting an indicator effect. It appears conceivable that at least a part of the observed coloration may have been due to the reaction of the alkali with the sugars that are certain to be present. One recalls the application of a somewhat analogous test to determine "diastatic activity," i.e., concentration of maltose, that was being made in certain German laboratories in 1937. Certainly fructose, if present, would react very readily, and one would expect to encounter it after boiling wheat meal, containing appreciable quantities of sucrose, in a solution of HCl in water.

After removing the carotinoids from flour with ether, Müller⁶² extracted the "bran pigments" (Kleiefarbstoffe) with 75 per cent alcohol. This extract was originally nearly colorless, and somewhat turbid because of the presence of proteins and mucilaginous materials. After the addition of ammonia, which developed the yellow color, and shaking with powdered infusorial earth, a clear solution was obtained. This was compared with a standard potassium dichromate solution.

One "yellow unit" was equivalent to a 0.030 per cent dichromate solution, .5 units = 0.073 per cent, 10 units = 0.224 per cent, 15 units = 0.682 per cent, and 20 units = 2.082 per cent. The flour extract represented 5 g flour in 10 cc of 75 per cent alcohol, to which 1 cc of 25 per cent ammonia solution was later added.

Müller examined a series of flour samples representing different degrees of extraction. Unfortunately he did not present any other data concerning the composition or properties of these flours. The median value, and the range in "yellow units" of these several flour types, classified in the Austrian system, is shown in Table 94.

Table 94. "Yellow Units" of Flours Examined by Müller⁶² Classified by Grades under the Austrian System.

Flour type	Yellow units	
	Median	Range
Ogg	3	1.5- 6
Og	5.5	4 - 8
Ogl	7	4 - 8
1	8.5	7 -10
2	9	6.5-10+
3	10.5	9.5-11.5
4	12	10 -13.5
5	13	12 -13+
6	14.5	12.5-16

It is the opinion of the present author that the endosperm of the wheat kernel has, as its principal pigment, a xanthophyll or xanthophylls of the lutein type, with small proportions of carotenes. The branny envelope evidently contains carotinoids, together with flavones and probably de-

⁶² Z. *Untersuch. Lebensm.*, 76, 225 (1938).

composition products of the chlorophylls as well. These last two are doubtless more soluble in alcohols, or alcohol-hydrocarbon mixtures than in hydrocarbons alone. Accordingly, they will be present as impurities in alcoholic extracts to an increasing degree as the proportion of bran in the flour or meal under examination is increased.

On the other hand, it appears probable that the carotinoids are not completely extracted from flour or wheat meal by a single extraction with a hydrocarbon or such hydrocarbon mixtures as are encountered in petroleum ether, or in naphtha. Accordingly, it may be assumed that an extract so recovered, while freer from non-carotinoid pigments, does not afford a fully quantitative measure of the concentration of carotinoids in the flour or meal.

Chapter 16

Vitamins of Wheat and Wheat Products

Such rapid progress is being made in these times in the field of vitamin research and technology that it is not possible to incorporate all of the significant contemporary work in a monograph of this type. Moreover, it is not the function of this monograph to present a general discussion of the discovery, constitution, and physiological functions of the several vitamins, or the levels of vitamin requirements of human and animal subjects. Numerous excellent texts are available to that end, and doubtless additional ones will appear annually, so long as the present rate of research in this field is maintained. Accordingly, the brief treatment here accorded the subject is confined rather definitely to the recorded data that pertain to wheat and its mill products. Even bread is omitted from consideration, for reasons indicated in the introduction.

Early in the period of vitamin investigations the vitamin A content of wheat was rated as poor by Steenbock and Coward,¹ which view has been generally held.

While his method of assaying was not highly quantitative, Hunt² did find that climate had an appreciable effect upon the vitamin B potency of wheat when the latter was estimated in terms of growth rate of rats. Hunt felt, moreover, that there was some indication that wheat plants fertilized with phosphates produced grain with an increased vitamin B content. The content of P or Ca in the grain did not parallel the vitamin B potency, however.

Ammonium sulfate used alone as a fertilizer, or in mixture with a complete mineral fertilizer, appeared to increase the vitamin B₁ content of wheat grown on the treated soil by the order of one-fifth over the control or unfertilized wheat in the experiments of Harris.³ Manure had no effect and a complete mineral fertilizer reduced the B₁ potency by about one-fifth. The bradycardia or heart-rate method of animal assaying was used in these tests. In terms of accuracy of the method used, the differences were not substantial—possibly not significant.

Another collection of samples from the same experimental station (Rothamsted Station, at Harpenden, England) was assayed later by

¹ *J. Biol. Chem.*, 72, 765 (1927).

² *Ohio Agr. Expt. Sta. Bull.* 415, 1-41 (1927).

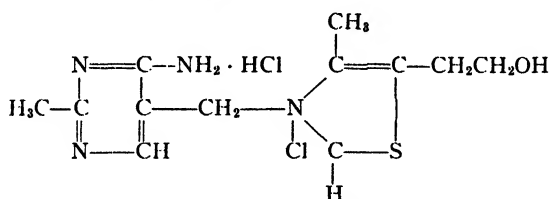
³ *J. Agr. Sci.*, 24, 410 (1934).

Leong⁴ using the same method, and he found that the vitamin B₁ content was not affected significantly by applications of manure, or by mineral mixtures, or ammonium sulfate. The wheats grown on the control, and the several fertilized plots contained from 1.0 to 1.3 International units of vitamin B₁ per gram.

Rating wheat germ as 100 in an arbitrary scale of vitamin B potency, Cramer and Mottram⁵ evaluated the products of milling as follows: middlings, 30; bran, 33; patent flour, 0.

Using the rat growth rate as a measure of the vitamin B₁ content of Kansas wheat and its mill products, the patent flour was found by Kramer⁶ to contain small but measurable amounts of the vitamin; first clear flour was about 40 per cent as rich as the wheat; low-grade flour 150 per cent; "middlings and germ stock" 400 per cent; and bran 250 per cent. Leavened bread made from whole-wheat flour contained four to five times as much B₁ as bread made from patent flour.

Using a rat growth method of assaying, Munsell and DeVaney⁷ found 4.5, 5.3, 6.7, and 10.0 Sherman units of vitamin B₁ respectively per gram in four samples of wheat germ. In a sample of soft winter wheat 1.5 Sherman units per gram was found.



Vitamin B₁, thiamine hydrochloride.
(aneurine)

In a summary of the B₁ content of foods published by Booher and Hartzler⁸ spring wheat was listed as having 525 μg of thiamine per 100 g (=2.38 mg per pound), hard winter wheat 477 μg per 100 g (=2.17 mg per pound), and soft winter wheat 354 μg per 100 g (=1.61 mg per pound). A "straight run" soft wheat flour milled by the Bureau of Plant Industry contained 87 μg of thiamine per 100 g (=0.395 mg per pound), a commercial packaged patent flour about 50 μg per 100 g (=0.23 mg per pound), and a white flour said by the producer to contain the germ portion of the wheat grain, 129 μg per 100 g (=0.59 mg per pound). It is evident that this germ-reinforced flour is far short of the vitamin content of any of the three wheat types.

⁴ *Biochem. J.*, **33**, 1397 (1939).

⁵ *Lancet.*, 1937, II, 1090.

⁶ Sixth Biennial Rpt. of the Director of the Kans. Agr. Expt. Sta. 113 (1932).

⁷ *Cereal Chem.*, **10**, 287 (1933).

⁸ U. S. Dept. Agr. Tech. Bull. 707 (1939).

These B₁ values were determined by the rat growth-rate method, and one International unit was regarded as equivalent to three micrograms (μ g) of thiamin.*

Straight flour representing 72 per cent by weight of the wheat was found by Atkin and Frey¹⁰ to contain 18 per cent of the total thiamine in that wheat. The bran and shorts each contained about 38 per cent, the germ only 5 per cent of the thiamine. When the straight-grade flour was divided into short patent (80 per cent), first clear (12 per cent), second clear (7 per cent) and red dog (1 per cent) these four flours contained the following percentages of the total thiamine of the straight grade: 42, 16, 27 and 21, respectively.

The actual concentrations of thiamine in these several mill products, in micrograms per gram, were: wheat 5.7; germ 30.0; bran 13.2; shorts 23.0; straight flour 1.5; short patent 0.7; first clear 1.8; second clear 5.1; red dog 21.2.

A summary of the determinations of the Vitamin B₁ content of wheats and mill products was prepared by Quagliariello,¹¹ and these data are recorded in Table 95.

One of the earliest comprehensive studies of the distribution of vitamin B in the wheat kernel was conducted by Bell and Mendel.⁹ This was published prior to the development of the present subdivision of the

* In view of the variety of unitages of thiamine used in the literature and carried in this chapter, the following table of equivalents may be useful:

1 μ g/g	=	0.10 mg/100 g 0.45 mg/lb 33.33 International units/100 g 151.32 International units/lb
1 mg/100 g	=	4.54 mg/lb 10.00 μ g/g 333.33 International units/100 g 1513.18 International units/lb
1 mg/lb	=	0.22 mg/100 g 2.20 μ g/g 73.42 International units/100 g 333.33 International units/lb
1 International unit/100 g	=	4.54 International units/lb 0.03 μ g/g 0.003 mg/100 g 0.0136 mg/lb
1 International unit/lb	=	0.22 International units/100 g 0.0066 μ g/g 0.00066 mg/100 g 0.0030 mg/lb

¹⁰ *Cereal Chem.*, 16, 643 (1939).

¹¹ *Quaderni nutriz.*, 6, 276 (1939).

⁹ *Am. J. Physiol.*, 62, 145 (1922).

Table 95. Vitamin B₁ Content of Wheat and Wheat Products, as Assembled by Quagliariello.¹¹

Material or product	Inter-national units per 100 g	Mg per 100 g	Reference
Wheat	170	0.34	Morgan, Hunt and Squire, <i>J. Nutrition</i> , 9, 395 (1935)
	152	0.30	Morgan and Hunt, <i>Cereal Chem.</i> , 12, 411
	No. 1 189	0.33	Morgan and Frederick, <i>Cereal Chem.</i> , 12, 390
	No. 2 142		
	250	0.50	Famiani, <i>Atti accad. Lincei</i> , 24, 88 (1936)
	285	0.57	Baker and Wright, <i>Proc. Roy. Soc. London</i> , 29, 1145 (1936)
	135	0.27	Scheunert and Schiebllich, <i>Biedermanns Zentr. (B)</i> 8, 120 (1936)
	208	0.42	Baker, Wright and Drummond, <i>J. Soc. Chem. Ind.</i> , 56, 191 (1937)
	118	0.24	Copping and Roscoe, <i>Biochem. J.</i> , 26, 1223 (1932)
	150	0.30	Leong and Harris, <i>Biochem J.</i> , 31, 812 (1937)
Flour, coarse	160	0.32	Baker, Wright and Drummond, <i>J. Soc. Chem. Ind.</i> , 56, 191 (1937)
Flour, coarse	150	0.30	<i>Ibid.</i>
Flour, 1st grade	110	0.22	<i>Ibid.</i>
Flour, 2nd grade	90	0.18	<i>Ibid.</i>
Flour, white	15	0.03	<i>Ibid.</i>
Flour, "Integrate"	80	0.16	De Caro, <i>Quaderni nutriz.</i> , 3, 171 (1936)
Flour, white 00	25	0.05	<i>Ibid.</i>
Flour, white	25	0.05	Leong and Harris (<i>loc. cit.</i>)
Flour, 94% extraction	120	0.24	Scheunert and Schiebllich (<i>loc. cit.</i>)
Flour, 82% extraction	100	0.20	<i>Ibid.</i>
Flour, 75% extraction	40	0.08	<i>Ibid.</i>
Flour, 60% extraction	24	0.05	<i>Ibid.</i>
Flour, 70% extraction	37	0.07	Copping and Roscoe (<i>loc. cit.</i>)
Flour, 42% extraction	0	0	<i>Ibid.</i>
Wheat germ	800	1.60	Chick and Jackson, <i>Biochem. J.</i> , 26, 1223 (1932)
	1090	2.18	Coward <i>et al.</i> † <i>Biochem. J.</i> , 31, 1879 (1937)
	700	1.40	Birch and Harris, <i>Biochem J.</i> , 28, 602 (1934)
	1230	2.46	Baker and Wright (<i>loc. cit.</i>)
	700	1.40	Morgan, Hunt and Squier (<i>loc. cit.</i>)
	770	1.54	Morgan and Frederick (<i>loc. cit.</i>)
	850	1.70	Morgan and Hunt (<i>loc. cit.</i>)
	600	1.20	Scheunert and Schiebllich (<i>loc. cit.</i>)
	720	1.42	Leong and Harris (<i>loc. cit.</i>)
	1250	2.50	Baker, Wright and Drummond (<i>loc. cit.</i>)
	1300	2.60	Baker and Wright, <i>J. Hyg.</i> , 37, 303 (1937)
Bran	130	0.26	Baker and Wright, <i>Biochem J.</i> , 29, 1802 (1935)
	300	0.60	Baker, Wright and Drummond (<i>loc. cit.</i>)
	360	0.72	Leong and Harris (<i>loc. cit.</i>)

* Probably an error. Should be *Biochem. J.*, 31, 1879 (1937).† Probably an error. Should be Copping *et al.*

vitamin B complex, and hence must be interpreted in terms of the nomenclature of knowledge of the early 20's. They did demonstrate that vitamin B is not confined to the embryo of the wheat kernel; in fact, their estimate of between 8 and 16 per cent of the total vitamin B of the kernel in the germ is in the range of present estimates for B₁. A concentration of vitamin B in the germ, equivalent to four to eight times that of the entire grain, was observed, however.

Endosperm was credited with 60 to 68 per cent of the total vitamin B of the kernel, and bran with approximately 24 per cent.

Scheunert of Leipzig¹² found an average of 130 I. U./100 g of Vitamin B₁ in wheat (=1.77 mg/lb) and 100 I. U. in rye (=1.36 mg/lb). These are lower values than are now being reported in American wheats. Rye contained more riboflavin than wheat, however, namely, 57.1 growth units of B₂ per 100 g (probably 171 µg/100 g or 0.777 mg/lb) against 50 units in wheat (=150 µg/100 g or 0.681 mg/lb). When wheat was converted into an 82 per cent extraction flour, the thiamine content of the latter was about 70 per cent of that of the original grain. When the flour recovery represented 60 per cent extraction, the thiamin content of the flour was 24 I. U./100 g, which is about 18.5 per cent of the concentration in the entire wheat. No substantial impairment of Vitamin B₁ in baking was observed.

Wheat flour representing 82 per cent extraction contained 33 growth units of Vitamin B₂, or 66 per cent of the amount in the entire wheat.

In a compilation of data concerning the occurrence of vitamins in foods, Munsell¹³ records 30 units (presumably the International unit equivalent to 3 µg of crystalline thiamine chloride) of Vitamin B₁ per 100 g of patent flour and 160 units per 100 g of whole wheat. This is the equivalent of 0.45 and 2.4 mg of B₁ per pound of patent and whole-wheat flour respectively.

The fermentation method of assaying for vitamin B₁ was employed by Schultz, Atkin and Frey¹⁴ in surveying American wheats and other cereals. A total of 31 wheat samples, including some pure varieties from the Minnesota, Kansas and Michigan Agricultural Experiment Stations, as well as commercial grades from Chicago. The average thiamine content was 5.6 µg per g (=2.54 mg per pound), and the range was from 4.2 to 7.3 µg per g (=1.91-3.31 mg per pound). While the number of samples is scarcely adequate for an analysis of the effect of environment or variety, there was some indication of trends observed in other instances toward higher thiamine concentration in the hard spring and *durum*

¹² *J. Am. Med. Assoc.*, 109, No. 13, 1054 (Sept. 25, 1937).

¹³ *Milbank Memorial Fund Quarterly*, 18, 311 (1940).

¹⁴ *Cereal Chem.*, 18, 106 (1941).

wheats, followed by hard winter and soft winter wheats in the order named.

Good agreement between the results of rat-growth assays and thiochrome determinations of thiamine in cereal products appeared in the studies of Andrews and Nordgren.¹⁵ They also observed that for many new cereal products the use of zeolite in the purification of extracts was unnecessary in the thiochrome analysis, since the effect of impurities was negligible. They recommended direct oxidation of extracts made by treatment with a 25 per cent KCl solution in dilute acetic acid for a rapid, routine thiamine estimation of enriched flour. Other raw cereal products gave only slightly lower values by this procedure than those obtained by the Hennessy and Cerecedo method. Bread was an exception, however, and treatment with a diastatic enzyme was required for complete evaluation of the vitamin B₁ content. This appeared to be due primarily to the presence of cocarboxylase which must be converted to free thiamine in the thiochrome analysis.

The thiamine content of spring wheats varied more as a function of environment than of variety, in the experiments of Nordgren and Andrews,¹⁶ although it is true that the varieties involved were of the same general type of strong bread wheats. Thus the averages of six varieties grown at four locations in Minnesota varied from 2.55 mg of B₁ per pound in Thatcher wheat to 2.90 mg per pound in Pilot wheat. On the other hand, the six varieties grown at St. Paul, Minnesota averaged 3.16 mg of B₁ per pound of wheat, while the same varieties grown at Crookston, Minnesota averaged only 2.24 mg of B₁ per pound. No trend toward a positive correlation between B₁ and protein content was evident in this series of wheat samples; the value of $r = +0.127$. There was a more definite relation between ash and B₁ content, however, the coefficient of correlation between these two variables being $r = +0.68$, and the 1 per cent point is 0.515 for the 24 pairs.

Winter wheats representing four common varieties, grown at four locations in the Great Plains Area, contained an average of 2.25 mg (corrected to 13.5 per cent moisture) of B₁ per pound. Another series of miscellaneous winter wheats averaged only 2.12 mg per pound. A collection of 15 soft winter wheats grown at a single location in the North Central States contained an average of 2.03 mg of B₁ per pound, and ranged from 1.79 to 2.38 mg. Two-thirds of the samples fell within the narrow range of 2.01 to 2.08, however. Spring and winter wheats grown in the Pacific Coast States averaged about the same as the miscellaneous hard winter wheats, namely 2.09 mg of B₁ per pound.

¹⁵ *Cereal Chem.*, **18**, 886 (1941).

¹⁶ *Cereal Chem.*, **18**, 802 (1941).

Among six varieties grown in Canada, the average of six hard spring (vulgate) wheats grown at Winnipeg was 2.62 mg of B₁ per pound, while the same varieties grown elsewhere in Canada averaged only 2.04 mg per pound. The average of the collection of 12 samples was 2.36 mg per pound, which was lower than the average of spring wheats grown in Minnesota. A single sample of Mindum *durum* wheat grown at Winnipeg contained 2.76 mg of B₁ per pound.

The B₁ content of corn (maize), rye, barley, and grain sorghums was in the same range as the hard wheats, while oats that were examined by these authors contained much more, namely 4.20 mg per pound.

The thiochrome method was applied by Booth¹⁷ to the determination of the thiamine content of 29 English wheats. These ranged from 0.78 to 1.98 I. U. per gram. Of the 29 samples, 17 or about three-fifths ranged between 1.2 and 1.6 I. U. per g. A considerable number of foreign wheats were also assayed and several samples of *durum* wheat were included in which the thiamine content was in excess of 3 I. U. per g. The Australian samples were also notable for their relatively high content of thiamine ranging from 1.50 to 2.60 I. U. per g and averaging 2.04 I. U. The following table affords a convenient indication of the distribution of these samples.

I. U. per g, range	No. of samples	Percentage of total
<1.0	13	25.5
1.01-1.40	21	41.2
1.41-1.80	10	19.6
1.81-2.20	4	7.8
>2.20	3	5.9
	51	100.0

The range in thiamine content of various cereals as recorded by Taylor¹⁸ is shown in Table 96.

The Council on Foods of the American Medical Association¹⁹ ac-

Table 96. Range in Thiamine Content of Various Cereals, as Compiled by Taylor.¹⁸

	Micrograms/lb
Wheat	1450-3800
Hard spring	1450-3490
Hard winter	1680-2710
Soft red	1790-2380
Pacific	1760-2440
Durum	2100-3800
Rye	1880-2280
Barley	2580-3330
Oats	2200-4900
Corn	1850-2280

¹⁷ *J. Soc. Chem. Ind.*, 59, 181T (1940).

¹⁸ *Wheat Studies of the Food Research Institute*, 18, No. 3, 77 (1941).

¹⁹ *J. Am. Med. Assn.*, 116, 2849 (1941).

cepted an average value of 2.04 mg of thiamine per pound for whole wheat. This is equivalent to 680 I.U./lb, or 0.45 mg/100 g, or 150 I.U./100 g. For white flour, the corresponding values were 0.23 mg/lb, or 77 I.U./lb, or 0.05 mg/100 g, or 17 I.U./100 g.

The following data were published anonymously²⁰ in an Australian journal:

	International Units of Vitamin B ₁ per gram
Whole wheat	1.8
Flours: 94% extraction	1.2
82% extraction	1.0
75% extraction	0.4
70% extraction	0.37
60% extraction	0.24

The increased thiamine content resulting from the extension of the extraction from 75 per cent, which is the equivalent of a straight-grade flour milled from plump, heavy wheat, to 82 per cent, which would include low-grade and tailings streams, is very striking.

Australian wheats were studied by Young, Wadham, Harris and Clements as members of a sub-committee of the Nutrition Committee of the (Australian) National Health and Medical Research Council.²¹ They found the average content of vitamin B₁ in 264 Australian wheats to be 760 I.U./lb (= 5.02 μ g/g = 2.28 mg/lb) with a range from 515 I.U./lb (= 3.40 μ g/g = 1.55 mg/lb) to 1,050 I.U./lb (= 6.92 μ g/g = 3.15 mg/lb). From sources that are not disclosed they accepted an average of 590 I.U./lb (= 3.89 μ g/g = 1.77 mg/lb) in English wheat. They also reported the value for American wheats that was accepted by the Council on Foods of the American Medical Association.¹⁹

Flour from every mill in Australia was assayed for its vitamin B₁ content, and upon statistical treatment of the data the true mean value lies between 1.56 and 1.79 μ g/g. They also estimated that 23.33 is the mean weighted percentage of Vitamin B₁ extracted from 100 pounds of wheat into 71 pounds of flour, with a standard error of ± 0.39 .

Using the thiochrome method of Wang and Harris, they found the standard error of duplicate determination to be ± 5.14 per cent. This means that in 95 per cent of the cases the mean obtained from duplicate determinations for a flour would lie within 2 times the standard error on either side of the true mean; that is, within 2 times $\pm 5.14\% = \pm 10.28$ per cent. In other words, the true mean would lie within ± 10 per cent of the actual mean of two analyses.

Among the principal mill products of eight flour mills, namely flour averaging 71.7 per cent, bran averaging 11.1 per cent, and "pollard"

²⁰ *Agr. Gaz. N.S. Wales*, 51, 377 (1940).

²¹ *Rpt. 12th Session Nat'l. Health and Research Council (Aust.)*, Appendix II (1941).

(equivalent of shorts in American terminology) these products contained the following percentages of the vitamin B₁ in the wheat from which they were milled: flour 26.1 per cent, bran 19.8 per cent, and pollard 54.1 per cent. The range in the percentage recovery in the flour was relatively wide, namely, 14.2 to 31.4 per cent. As has been observed in American mills, the highest content of B₁ was encountered in the flours from the reduction rolls at the end or tail of the system.

Rat assays were made by Biswas²² in determining the vitamin B₁ content of whole wheat flour or *ata*, and white flour. In terms of Guha and Chakravorty units the whole wheat flour contained 50.3 units of vitamin B₁ and the white flour 68.5 units. These units are apparently a special value unlike the International units generally employed in Europe and America. Moreover, the relative levels of B₁ thus recorded are quite contrary to those commonly observed for whole wheat and white flour, although again this depends upon what is meant by *white* flour.

Canadian hard spring wheats of the 1940 crop loaded from Manitoba, Saskatchewan and Alberta (Canada) shipping points, in the number of 265 samples, were subjected by Johansson and Rich²³ to the determination of their thiamine content. For this purpose the thiochrome method, as modified by these investigators, was used. A summary of their data appears in Table 97. No substantial differences between the wheats from three provinces are apparent except that the range and the standard deviation are a little greater in the instance of the Alberta samples. In general, the averages are in the same range as were reported for world wheats by Booth.¹⁷

When the individual thiamine data were placed upon a map in the area of the origin of the sample they were scattered too heterogeneously to permit drawing any definite conclusions respecting the effect of environment, including soil, upon the deposition of thiamine in the wheat kernel.

No correlation between ash content and thiamine content, or between protein content and thiamine content was apparent. Thus the coefficients of correlation in these two sets of comparisons were: $r = +0.012$, and $r = +0.161$, respectively. The 5 per cent. in both cases was ± 0.195 . Neither was there any observed relation between thiamine content and weight per bushel or grade, but the series of samples were not singularly adapted to such a comparison, since the lots were fairly uniformly plump and of good quality.

One lot was hand-picked to separate the plump and the shrunken kernels, and these were found to contain 3.4, and 4.2 μg of thiamine per

²² *Science and Culture*, 6, 245 (1940); *Chem. Abs.*, 35, 1097 (1941). Original not seen.

²³ *Cereal Chem.*, 19, 308 (1942).

gram of wheat respectively. Starchy kernels from one lot contained only 2.9 μg of thiamine per g, whereas the "normal" kernels from the same lot contained 3.5 μg per g.

Table 97. Average Thiamine Content of Canadian Spring Wheats of the 1940 crop, Arranged by Provinces, as Reported by Johansson and Rich.²³

Province	No. of samples	Micrograms per g on 13.5% moisture basis		
		Range	Mean	Standard deviation
Manitoba	67	3.0-5.3	3.90	0.606
Saskatchewan	120	2.5-6.2	3.99	0.708
Alberta	78	2.2-8.0	3.90	0.909
All three provinces	265	2.2-8.0	3.93	0.750

The thiamine content of two collections of hard and soft wheats respectively was determined by Conner and Straub,²⁴ using the method previously described by Conner⁴² for the combined determination of these two vitamins. Thiamine in the 15 hard wheat samples ranged from 3.65 to 6.90 μg per g and averaged 5.03. The 16 soft wheats ranged from 2.43 to 4.77 $\mu\text{g/g}$ in thiamine content and averaged 3.52. This tendency toward a higher thiamine content in hard wheats has been encountered elsewhere.

Wheat germ was found to contain 30-45 $\mu\text{g/g}$, wheat bran 19-33 $\mu\text{g/g}$ of thiamine.

Within a wheat variety, there was evidence of considerable variation in thiamine content as a function of environment during the growing period.

The examination of 118 samples of wheat germ drawn from eleven different sources of supply by Baker and Wright²⁵ revealed a five-fold variation in vitamin B₁ content. The germ samples were cleaned by removing all bran, middlings and flour, and accordingly the differences in vitamin content could not be attributed to variations in the proportion of impurities present. The same method of assay, involving the cure of induced bradycardia was employed throughout, and with an observed accuracy of ± 20 per cent. Three samples containing 4 units of B₁ per g were encountered, and two samples with 22 units per gram. Of the 118 samples, 58 per cent contained between 10 and 16 units per g and the modal value was in the range of 13 units per g.

Evidence of varietal differences in the thiamine content of Canadian spring wheats appears in the data of Tisdall, Jackson, Drake, Newman, Whiteside, Miller and Edgar.²⁶ Thus two of the new rust-resisting wheat varieties, Regent and Renown, were outstandingly high in their content

²⁴ *Cereal Chem.*, 18, 671 (1941).

²⁵ *J. Hyg.*, 37, 303 (1937).

²⁶ *Can. Med. Assoc. J.*, 45, 101 (1941).

of thiamine, as was also the variety Reward. Garnet, on the other hand, was decidedly low in thiamine. The averages of their assays, involving wheats grown at 158 points in the western hard spring wheat area of Canada in the instance of the last five varieties are shown in Table 98.

Table 98. Thiamine Content of Several Varieties of Canadian Wheat, as Reported in International Units per Pound by Tisdall, *et al.*²⁶ and Calculated by the Author into Terms of Micrograms per Gram, and Milligrams per Pound.

Variety	I. U. per lb	Thiamine content	
		Calculated as $\mu\text{g/g}$	mg/lb
Garnet (Lacombe, Alberta)	456	3.01	1.37
White Fife (Charlottetown, P.E.I.)	476	3.15	1.43
Marquis	645	4.26	1.93
Thatcher	700	4.62	2.10
Reward	806	5.32	2.41
Renown	820	5.42	2.46
Regent	820	5.42	2.46

Using a fermentation method Downs and Cathcart²⁷ determined the thiamine content of over 100 wheat samples of a number of market classes. Hard wheats had a higher average thiamine content, 7.1 μg per g, than the soft wheats, which averaged 6.1 μg per g. The hard wheats were quite variable in their thiamine content, as evidenced by the distribution table (Table 99) computed by the author from the data presented by Downs and Cathcart.

Table 99. Distribution, in Percentage of Samples Tested, of the Thiamine Content of all Hard Wheat Samples Assayed by Downs and Cathcart.²⁷

Thiamine content ($\mu\text{g/g}$) (range)	Percentage of the samples tested
4.0- 5.0	4.8
5.1- 6.0	21.3
6.1- 7.0	25.9
7.1- 8.0	32.7
8.1- 9.0	10.6
9.1-10.0	3.8
10.1-11.0	1.0

The same investigators also reported the average thiamine content of the several market classes of wheat, as recorded in Table 100. The vitreous types, such as *durum*, hard winter and spring, appeared to contain more thiamine than the softer types.

Thiamine was determined in (Indian) wheat and flour by Shourie and Sundararajan²⁸ by the thiochrome method. Whole wheat and whole-wheat flour contained 4.0-4.2, white flour 1.2, and semolina 1.5 μg per g, respectively.

²⁷ *Cereal Chem.*, 18, 796 (1941).

²⁸ *Indian J. Med. Res.*, 30, 61 (1942).

Table 100. Average Thiamine Content of Wheat Samples by Market Classes, as Reported by Downs and Cathcart.²⁷

Market class	No. of samples	Average thiamine content μg/g
Spring	10	6.5
Hard winter	70	7.2
Soft winter	17	6.2
Western red	2	6.9
White wheat, including "club"	13	6.0
Durum	9	7.3

In the estimate of Sherwood, Nordgren and Andrews,²⁹ the endosperm of the wheat kernel contains about 24 per cent of the total thiamine of the wheat kernel, the embryo about 15 per cent, and the outer layers about 61 per cent. Three sets of mill products from one commercial mill were subjected to analysis for their thiamine content by the thiochrome method, and the results are given in Table 101. It is evident that the

Table 101. Thiamine (Vitamin B₁) Distribution in Products of Three Commercial Wheat-milling Operations in One Mill, as Reported by Sherwood, Nordgren and Andrews.²⁹

Sample	Milling yield of cleaned wheat (%)	Thiamine content	
		Mg per pound*	Calculated as per cent of total thiamin in wheat
Patent flour	63.0	0.31	8.0
First clear flour	7.0	1.36	3.9
Second clear flour	4.5	5.61	10.0
Red dog flour	4.0	13.45	22.0
Germ	0.2	10.40	0.9
Shorts	12.3	7.89	39.6
Bran	9.0	4.25	15.6
Cleaned wheat	100.0	2.28	100.0

*1 mg per lb=0.74 I. U. per g.

streams which make up the Red Dog Flour must be very rich in B₁, which accounts for the large increase in the relative or percentage return of thiamine when a composite, extended extraction flour is produced in the manner suggested by Table 102.

Table 102. Relation of Per Cent Extraction to Per Cent of Total Thiamine in Cleaned Wheat; Theoretical Values Obtained by Calculation, Not by Actual Production of Long-extraction Flour, as Reported by Sherwood, Nordgren and Andrews.²⁹

Sample	Extraction of cleaned wheat (%)	Total thiamine in cleaned wheat (%)
1. Patent flour	63	7.8
2. Patent flour plus first clear	70	11.8
3. (2) plus second clear	74.5	23.8
4. (3) plus red dog	78.5	48.8
5. (4) plus germ and shorts	91	85.4
6. (5) plus bran (entire wheat)	100.0	100.0

²⁹ *Cereal Chem.*, 18, 811 (1941).

In addition to the three sets of samples from one mill, the thiamine assays of which are recorded in Table 101, Sherwood, Nordgren and Andrews presented the means and the range in thiamine content of analogous streams from nine mills situated in widely separated regions of the United States. These data are recorded in Table 103, together

Table 103. Thiamine and Ash Content of the Products of Commercial Wheat Milling, as Conducted in Nine Widely Separated Flour Mills (Data of Sherwood, Nordgren and Andrews²⁹).

Sample	(mg per lb*) Thiamine		Ash	
	Average	Range	Average (%)	Range (%)
Cleaned wheat	2.20	1.97- 2.43	1.56	1.40-1.75
Patent flour	0.32	0.23- 0.48	0.40	0.36-0.43
First clear	1.20	0.50- 1.66	0.68	0.44-0.85
Second clear	3.76	2.00- 5.34	1.33	0.88-2.34
Red dog	11.74	10.39-13.45	2.83	2.44-3.70
Shorts	8.03	5.60- 9.83	4.35	3.63-5.16
Bran	3.99	2.76- 4.98	6.52	6.30-6.94

*All samples contained about 11 per cent moisture at time of analysis.

with the percentage of ash. It is evident that wide variations in thiamine content may be encountered in mill products bearing the same commercial designation. This may be a function in part of the properties of the wheats from which the products are milled, but more probably the variation is occasioned by the differences in milling systems and practices as reflected in the relative partition of the high vitamin tissues among the several end products.

The scutellum portion of the wheat germ was found by Hinton³⁰ to contain about ten times as much vitamin B₁ as the "embryo." Hinton's use of the term "embryo" in this connection is different from American usage, which latter is exemplified by Winton and Winton's³¹ diagram in which he includes the scutellum as part of the embryo. Hinton, on the other hand, considers the embryo to comprise only the plumule and the radical portions exclusive of the scutellum. The embryo, thus defined, was found to constitute 1.2 per cent by weight of English wheat, the scutellum 1.5 per cent. Thus described, the vitamin B₁ content of the scutellum was estimated at 40 I.U. per g, that of the embryo at 4 I.U. per g. There was no material difference in the riboflavin content of the two structures, however, and the average content was in the range of 15 μ g per g.

When wheat kernels were degermed in a scourer by Ward,³² the degermed fraction had a much lower concentration of vitamin B₁ than the embryos that were recovered. The ratio was about 1:6 as shown here:

³⁰ *J. Soc. Chem. Ind.*, 61, 143 (1942).

³¹ "Structure and Composition of Foods," p. 191, New York, 1932.

³² *Chem. and Ind.*, 62, #2, 11 (1943).

	Wt. (%)	B ₁ (I. U./g)	Total Contribution
Degermund kernels	97½	0.62	0.60
Embryo	2½	3.95	0.10
Entire wheat	100	1.30	

When the kernels were divided into two halves most of the B₁ appeared in the germ half, as follows:

	Wt. (%)	B ₁ (I. U./g)	Total Contribution
Beard end	46.5	0.36	0.167
Germ end	53.5	2.09	1.120
Entire wheat	100.0	1.29	

Studies of flour-mill stocks showed that there was a rich source of B₁ either in the outer layers of the embryo or in cells adhering to it. A further dissection of the kernels was then made which disclosed that the two fractions designated as C and D below, and described as scutellum, and epithelium with some scutellum contained B₁ in a concentration about seven times as great as the fraction B described as "embryo plant." The latter comprised chiefly the radical and plumule portions of the wheat embryo. The data upon which these conclusions were based are as follows:

	Wt. (%)	B ₁ (I. U./g)	Total Contribution
Degermed wheat (E)	97.43	0.61	0.594
Embryo plant (B)*	1.04	6.36	0.066
Bran covering embryo (A)	0.33	2.95	0.010
Scutellum (C)	0.63	49.4	0.311
Epithelium with some scutellum (D)	0.54	42.3	0.228
Whole wheat	99.97		1.209

* Chiefly the radical and plumule.

It thus appears that the centers of B₁ concentration in the wheat kernel are the scutellum and adjacent epithelial layers, although the quantity of B₁ in degermed wheat is large because it represents the bulk of the kernel.

Ward's findings and conclusions appear to be in general harmony with those reported by Hintcn.³⁰

The principal products of a Canadian flour mill were found by Alcock and Larmour³³ to contain widely varying concentrations of thiamine as follows:

	Percentage of wheat	Thiamine (μg/g)
Flour	75	1.43
Low-grade flour	2	14.00
Total flour	77	1.75
Shorts	12	21.00
Bran	11	8.00
	100	4.75

³³ *Canadian Chem. and Process Ind.*, 26, 3 (1942).

They classified the flour streams in this flour mill into four major groups; these were assayed separately and found to contain the following amounts of thiamine:

	Percentage of wheat	Thiamine ($\mu\text{g/g}$)
First-grade flour	39	0.9
Second-grade flour	30	1.6
Third-grade flour	6	4.0
Low-grade flour	2	14.0
	<hr/> 77	1.75

Using the thiochrome method for the determination of thiamine Shetlar and Lyman³⁴ analyzed the flour streams from a five-break, seven-reduction flour mill grinding commercial Ohio soft wheat. Their results, together with like determination of thiamine in the red dog, shorts, and bran, are recorded in Table 101. Preliminary studies showed a limited effect from the inclusion of a treatment of the digest with takadiastase in the instance of the flours, including the red dog, which suggested that this treatment may be unnecessary with such material. In the instance of the shorts sample, the omission of the takadiastase treatment reduced

Table 104. Distribution of Thiamine in the Several Mill Streams from a Five-break, Seven-reduction Flour Mill Grinding Commercial Ohio Soft Wheat, as Reported by Shetlar and Lyman.³⁴

Laboratory number	Stream	—Thiamine ($\mu\text{g per g}$)—		Ash content 13.5% moisture basis (%)
		Found ¹	Corrected (times 1.11)	
1179	1st break flour	0.8	0.9	0.404
1180	2nd break flour	1.0	1.1	0.406
1181	3rd break flour	0.8	0.9	0.474
1182	4th break flour	0.7	0.8	0.480
1183	5th break flour	1.3	1.4	0.590
1184	1st mids flour	0.9	1.0	0.338
1185	2nd mids flour	0.6	0.7	0.350
1186	4th mids flour	0.8	0.9	0.376
1187	5th mids flour	1.3	1.4	0.424
1188	6th mids flour	2.0	2.2	0.478
1189	7th mids flour	2.4	2.6	0.648
1190	1st tailings flour	1.1	1.2	0.535
1191	2nd tailings flour	1.8	2.0	0.560
1192	Sizings flour	0.7	0.8	0.326
1193	Bran and shorts dust reel	2.4	2.6	0.740
1194	1, 2, 3 breaks, hex. reel cuts	1.2	1.3	0.840
1195	Low-grade flour	3.6	3.9	0.684
1196	3rd mids patent flour	0.7	0.8	0.328
1197	3rd mids clear flour	0.9	1.0	0.358
1198	Red dog	9.2	10.1	—
1199	Shorts	9.3	10.2	—
1200	Bran	5.2	5.7	—

¹ At least two analyses on different days, checking within 10 per cent of each other, were used in obtaining the average results.

³⁴ *Cereal Chem.*, 18, 666 (1941).

the recovered thiamine by about 40 per cent. Since only about 88-91 per cent of added thiamine was accounted for by the method which they employed, Shetlar and Lyman corrected all their findings by multiplying by the factor 1.11; these corrected values, together with the actual findings, are recorded in Table 104. The ash content of the flour streams, corrected to 13.5 per cent moisture basis, is also shown.

In general, there was a trend toward an increasing thiamine content with increasing ash content, although the correlation was far from perfect. Secondary middlings (6th, and 7th) 2nd tailings, and the duster reel streams tended to be high in thiamine while, as in the instance of other recent studies, the red dog was unusually rich.

Distribution of thiamine in the products of flour milling is dependent upon the type of milling process, as disclosed by the observations of Schultz, Atkin and Frey.³⁵ Thus stone-ground flour of 62 per cent extraction contained 4.52 $\mu\text{g/g}$ ($= 2.03 \text{ mg/lb}$) of thiamine, whereas roller-mill flour of 63 per cent extraction was found by Sherwood, Nordgren and Andrews²⁹ to contain only 0.31 mg/lb, and a 60 per cent extraction flour examined by Scheunert contained 0.33 mg/lb. The average for roller-ground patent flour reported by Munsell was 0.45 mg of thiamine per pound, which is approximately double the concentration accepted by the Council on Foods of the American Medical Association,³⁶ namely, 0.23 mg/lb. Two other values reported by Schultz, *et al*, for stone-ground flours were 4.04 $\mu\text{g/g}$ ($= 1.82 \text{ mg/lb}$) for a 54 per cent extraction flour from a second mil, and 4.74 $\mu\text{g/g}$ ($= 2.13 \text{ mg/lb}$) for a 55 per cent extraction flour from a third mill. While the results from any given system of milling are not in perfect agreement, it does appear evident that the stone-ground flours carry a much larger proportion of the thiamine of the wheat than flours milled by the more modern roller-milling process.

At a conference held in Ottawa, Canada, on July 30, 1941 White-side³⁷ reported that the several thirds of the wheat kernel contained the following quantities of vitamin B₁.

Third of kernel represented by	I. U. of B ₁ per lb
Germ end	1700
Center section	158
Brush end	222

Thus more than 80 per cent of the B₁ content of the entire kernel was found in the germ end, and since the germ itself represents only about 2½ per cent or less of the kernel by weight, it follows that the non-germ

³⁵ *Cereal Chem.*, 19, 529 (1942).

³⁶ *J. Am. Med. Assn.*, 116, 2849 (1941).

³⁷ Mimeographed report entitled "Conference of Millers and others interested in high vitamin flour, Chateau Laurier, Ottawa, July 30, 1941," p. 6.

portion of the lower third of the kernel must be exceedingly rich in thiamine.

These observations were confirmed by Andrews,³⁸ who found the germ half of the wheat kernel to contain 4.76 mg/lb of thiamine, whereas the other or brush half contained only 0.82 mg/lb. Thus the ratio between the two halves is 5.80:1.00.

Commercial Canadian hard wheat samples assayed for thiamine content by Jackson and Whiteside³⁹ were found to contain from 637 I.U./lb to 735 I.U./lb ($= 4.20 \mu\text{g/g}$ to $4.85 \mu\text{g/g}$). An examination of 10 cargoes each of Manitoba 1 Northern, 2 Northern, 3 Northern and 4 Northern (Canadian Spring) wheat did not disclose any substantial difference between the four grades. The average of this collection of 40 cargoes was 660 I.U./lb ($= 4.35 \mu\text{g/g}$). Garnet wheat cargoes were rather uniformly lower in thiamine, however, the average of eleven cargoes being 553 I.U./lb ($= 3.65 \mu\text{g/g}$). Among six common varieties of hard spring wheat grown in Canada, Regent, Renown, and Reward were outstandingly high in thiamine content, averaging 815 I.U./lb ($= 5.35 \mu\text{g/g}$); Thatcher, Marquis and Apex were lower, averaging 672 I.U./lb ($= 4.43 \mu\text{g/g}$). In another series of comparisons of six varieties grown at 5 different locations, Garnet averaged lowest, Regent highest in thiamine content, and with indications of a tendency toward a place effect although the results were not very uniform nor conclusive.

Flour streams from two different mills were also subjected to thiamine assays by Jackson and Whiteside³⁹ and, as disclosed in other related studies, the primary, refined middlings streams were lowest in thiamine, ranging around 60 to 160 I.U./lb, whereas low-grade, duster and reel streams contained between 600 and 1110 I.U./lb. The latter represented a small percentage of the production of the mills, however. In a study of the relation between moisture content of the wheat and thiamine content of the straight-grade flour, there was evidence of a tendency toward a higher thiamine content when dry wheats (12.5 per cent moisture) were milled. The differences in thiamine content between flours milled from wheat containing 15.5 per cent moisture and 12.5 per cent moisture averaged in the range of about 45 per cent based upon the high-moisture wheat flour.

Average bakers' patent flour milled in Canada contained 160 I.U./lb ($= 1.06 \mu\text{g/g}$).

Cereals appeared to contain a cocarboxylase, according to Maltha,⁴⁰ which is measured by the yeast fermentation, but not by the thio-

³⁸ Address before the Minnesota Institute of Cereal Chemistry, Feb. 26, 1943.

³⁹ *Sci. Agr.*, 22, 386 (1942).

⁴⁰ *Chem. Weekblad*, 38, 555 (1941).

chrome method for determining thiamine. Thus a 75 per cent extraction flour containing 0.61 per cent of ash returned a value of 3.6 $\mu\text{g/g}$ of thiamine or "aneurine" as determined by the fermentation method, which appeared to be considerably higher than the values generally accepted for such flour. A series of flours of varying extraction were then tested by the two methods and with the following results:

Percentage extraction	100	85	82	80
Ash content (%)	1.65	1.06	0.98	0.90
"Aneurine," fermentation method ($\mu\text{g/g}$)	6.6	5.4	5.2	4.1
"Aneurine," thiochrome method ($\mu\text{g/g}$)	3.25	3.2	2.5	2.5

The total thiamine content of the entire spring sown wheat plant above ground ("tiller") apparently had reached a maximum at or shortly after blossoming, in the investigation conducted during the one crop season of 1940 by Geddes and Levine.⁴¹ Moreover, it tended to remain stationary in amount or even diminish slightly as maturation progressed. The proportion of thiamine in stems, glumes and kernels changed very decidedly during the period from the 7th to the 31st day after blossoming, however. Thus in the instance of the early-sown Thatcher wheat, only 25 per cent of the total thiamine of the plant was in the kernels on the 7th day, while on the 31st day, when fully ripe, 77 per cent was in the kernels. This increase apparently was at the expense of the glumes and stems, which lost thiamine proportionately.

The actual quantity of thiamine in the kernels increased as they developed, but the concentration in micrograms per gram did not change substantially between the 15th and the 31st days after blossoming; it was 6.0 μg per g on the 15th day, and 6.5 on the 31st day. These changes in concentration, amount, and distribution in the several structures of the wheat plant as observed by Geddes and Levine³⁹ are shown graphically in Figure 16.

The content of certain vitamins in wheat was reported by Conner⁴² as follows: Thiamine 87-195 $\mu\text{g/oz}$ (or 1.39-3.12 mg/lb); riboflavin 19.48 $\mu\text{g/oz}$ (or 0.30-0.77 mg/lb); nicotinic acid 0.85-1.30 $\mu\text{g/oz}$ (or 13.6-20.8 mg/lb); pantothenic acid, and pyridoxin +. These concentrations are greater than those of corn and rice, but less than that of oats in the instances of thiamine and riboflavin.

Malted wheat had a slightly lower thiamine content than unmalted wheat in the experiments conducted by Davis, Laufer and Salatan.⁴³ Thus the five unmalted wheats used by them contained an average of 5.68 $\mu\text{g/g}$ of thiamine, the malted wheats 5.02 $\mu\text{g/g}$. Nicotinic acid and

⁴¹ *Cereal Chem.*, 19, 547 (1943).

⁴² *Ind. Eng. Chem.*, 33, 711 (1941).

⁴³ *Cereal Chem.*, 20, 109 (1943).

pantothenic acid increased slightly in malting. Thus the average nicotinic acid content of five unmalted wheats was $58.6 \mu\text{g/g}$, and of the malted wheats, $67.3 \mu\text{g/g}$. Also the average pantothenic acid content was 7.9, and $9.0 \mu\text{g/g}$ of the unmalted and the malted wheats respectively. In the instance of riboflavin, malting increased the concentration of this vitamin more than three-fold on the average, *i.e.*, from $0.91 \mu\text{g/g}$ in the unmalted, to $2.91 \mu\text{g/g}$ in the malted wheat.

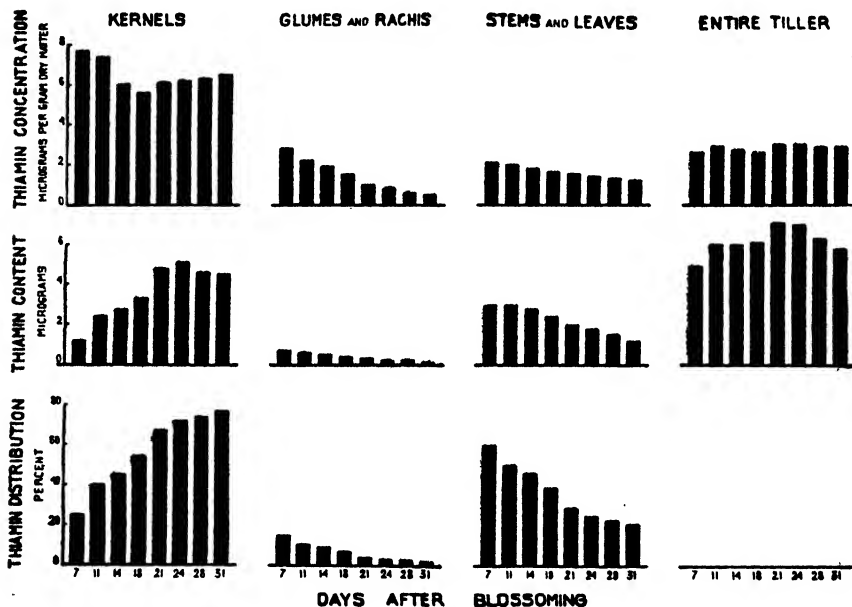


FIG. 16. Diagrams showing the concentration, content and distribution of thiamine in different fractions of the wheat plant at successive stages of kernel development as reported by Geddes and Levine.⁴¹

Using the Sherman and Bourquin method of assaying, Munsell and DeVaney⁴⁴ found 1.8, 2.3, 2.6, and 3.8 Sherman units of Vitamin B₂ per gram in the instance of four samples of wheat germ. It chanced that the sample which contained the most Vitamin B₁ was lowest in Vitamin B₂. In a sample of soft winter wheat they found 0.8 Sherman unit of Vitamin B₂ per gram of wheat. This was about equal to the sample of rice polishings which they assayed.

A fluorescent method for the determination of riboflavin developed by Murthy⁴⁵ was applied by him to forty foodstuffs, including several cereals. Whole wheat was reported to contain 1.19, white maize, 3.09, un-

⁴⁴ *Cereal Chem.*, 10, 287 (1933).

⁴⁵ *Indian J. Med. Res.*, 24, 1083 (1937).

milled rice 1.22, and polished rice 0.8 $\mu\text{g/g}$. The value here reported for whole wheat is in the same range as the concentrations later determined in American wheats by microbiological techniques.

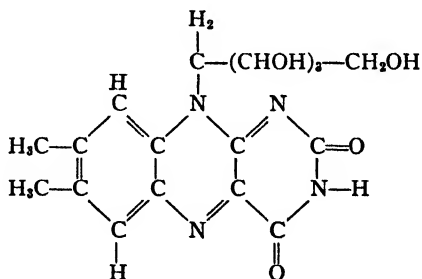
Whole wheat was found by Munsell⁴⁶ to contain 80 to 100 $\mu\text{g}/100\text{ g}$ of riboflavin. She also recorded the assays of Wilson and Roy,⁴⁷ who found 37 and 40; Hodson and Norris⁴⁸ who found 80; and Lunde, Kringstad and Olsen⁴⁹ who found 140.

A compilation of earlier determinations of the riboflavin content was arranged by Quagliariello¹¹ which has been included here as Table 105.

Table 105. Riboflavin Content of Wheat Products, as Assembled by Quagliariello.¹¹

Material	per 100 g		Author and citation
	Rat units	Milli-grain	
Wheat	32	(0.13)	Roscoe and Ackroyd,* <i>Biochem. J.</i> , 23, 184 (1939)
	(5)	0.02	Euler, Adler and Schlötzer, <i>Z. physiol. Chem.</i> , 226, 87 (1934)
	20	(0.08)	Birch, György and Harris, <i>Biochem. J.</i> , 29, 2830 (1935)
	20	(0.08)	De Caro, <i>Quaderni nutriz.</i> , 3, 171 (1936)
	50	(0.20)	Scheunert and Schieblich, <i>Biochem. Z.</i> , 290, 398 (1937)
Flour, fine	20	(0.08)	De Caro (<i>loc. cit.</i>)
" , 82%	33.3	(0.13)	Scheunert and Schieblich (<i>loc. cit.</i>)
" , 60%	11.1	(.045)	Scheunert and Schieblich (<i>loc. cit.</i>)
Germ	(8)	0.033	Kuhn, <i>Ber.</i> , 67, 1452 (1934)
	100	0.40	Birch, György and Harris (<i>loc. cit.</i>)
	250	(1.00)	Scheunert and Schieblich (<i>loc. cit.</i>)
Bran	100	(0.40)	Birch, György and Harris (<i>loc. cit.</i>)

*Probably an error. Should be Aykroyd and Roscoe, *Biochem. J.*, 23, 483 (1929).



Vitamin B₂, riboflavin, 6,7 dimethyl-9-*d*, 1-ribityl-isoalloxazine
(Vitamin G, lactoflavin)

After extracting both thiamine and riboflavin from the ground food material with 0.04*N* sulfuric acid, Connor and Straub⁵⁰ adsorbed the

⁴⁶ *Food Research*, 7, 85 (1942).

⁴⁷ *Indian J. Med. Res.*, 25, 879 (1938).

⁴⁸ *J. Biol. Chem.*, 131, 621 (1939).

⁴⁹ *Nord. Med. Ark.*, 3, 2583 (1939).

⁵⁰ *Ind. Eng. Chem., Anal. Ed.*, 13, 385 (1941).

former on Decalso, and the latter on Supersorb. The riboflavin was eluted from the Supersorb with a solution of 20 per cent pyridine in 2 per cent acetic acid, this solution treated with potassium permanganate, followed by hydrogen peroxide, and observed in a fluorophotometer, using a Corning glass filter No. 511 for transmitting the incident light, and No. 351 for the fluorescent light. Results obtained by this method were in good agreement with those obtained by bioassay. When thus tested, wheat was found to contain 0.89 to 2.03 μg per g of riboflavin, wheat germ 3.78 to 5.56, and wheat bran 3.17. These results are in agreement with Andrews *et al.*,³² so far as wheat and bran are concerned, but are appreciably lower in the instance of the germ. It must be recognized that commercial wheat germ is quite variable in purity; accordingly, there is no basis here for directly comparing the adequacy of the method employed.

Two series of wheats, including 15 samples of hard wheats and 16 samples of soft wheat were analyzed for their riboflavin content by Conner and Straub,⁵¹ using the fluorometric method previously described by them.⁵⁰ The hard wheats averaged 1.17 μg of riboflavin per g; they ranged from 0.89 to 1.91 μg , but it should be emphasized that the maximum figure is not of primary significance as evidencing trends, since the second in order of riboflavin content contained considerably less, namely 1.41 $\mu\text{g/g}$. The soft wheats averaged 1.07 $\mu\text{g/g}$, and ranged from 0.81 to 1.48 μg . Thus there is less difference, relatively, in the average riboflavin content of the hard and soft wheats than was observed in the thiamine content. Also variety and environmental conditions under which the wheat was grown appeared to exercise less effect upon riboflavin content than was observed in the instance of thiamine content.

Wheat germ was found to contain 4-5 $\mu\text{g/g}$, wheat bran 3-4 $\mu\text{g/g}$ of riboflavin.

The riboflavin or vitamin B₂ content of the same series of 24 spring wheat samples to which reference is made in the section on thiamine, was determined by Andrews, Boyd and Terry,⁵² who employed the microbiological method of Snell and Strong. It was found necessary to employ the use of takadiastase in preparing samples for analysis in order to overcome the influence of non-flavin growth-stimulating factors. With this technique riboflavin values obtained by both microbiological and fluorometric methods were in fairly satisfactory agreement. The average riboflavin content of these spring wheats was 1.20 μg per g.

In contrast to the observations made on thiamine, no influence of environment was apparent in this series. Thus the averages of six varieties at the four stations were: St. Paul, Minn., 1.21; Morris, Minn.,

⁵¹ *Cereal Chem.*, **18**, 671 (1941).

⁵² *Cereal Chem.*, **19**, 55 (1942).

1.23; Waseca, Minn., 1.21; and Crookston, Minn., 1.17 μg per g. On the other hand there seemed to be a distinct varietal difference, Marquis wheat containing consistently about 25 per cent more riboflavin than the lowest variety, Rival. The averages for the four stations by varieties, in μg of riboflavin per g of wheat were:

Marquis	1.32	Ceres	1.20
Pilot	1.24	Renown	1.17
Thatcher	1.21	Rival	1.06

The products resulting from the roller milling of wheat were also tested for their riboflavin content by both a microbiological, and a fluorometric method, with the results shown in Table 106. The thiamine content of the same samples was also determined. While the patent flour

Table 106. Riboflavin and Thiamine Content of Products of Milling, as Reported by Andrews, Boyd and Terry.⁵⁸

Mill product	Percentage of wheat	Thiamine ($\mu\text{g/g}$)	Riboflavin ($\mu\text{g/g}$)	Percentage of total vitamin of wheat in each mill product	
				Thiamine	Riboflavin
Wheat	100.0	4.41	1.00	100.0	100.0
Patent flour	65.0	0.84	0.34	12.1	20.5
1st clear	5.5	3.39	0.62	4.1	3.2
2nd clear	4.5	18.66	1.85	18.5	7.7
Red dog	4.0	27.09	3.80	24.0	14.4
Shorts	12.5	9.84	2.80	27.2	32.5
Bran	8.5	7.50	2.80	14.1	22.0

was low in riboflavin content the large yield of this product of milling resulted in a recovery in the patent flour of about one-fifth of the total riboflavin in the grain. The content of this vitamin reached the highest level in the red dog as was true of the thiamine. The relative variability among the various mill products was not as great in the instance of riboflavin as appeared in the thiamine content. Shorts and bran followed in that order, insofar as concentration of riboflavin per gram was concerned, but in terms of actual quantity, these mill products contained much of the riboflavin in the original wheat, namely, over 55 per cent.

From these data graphs were presented later by Andrews, Boyd and Gortner⁵⁸ that are included in Figure 17 and indicate the percentage of the riboflavin as well as the thiamine and nicotinic acid of the wheat that is recovered in the flour or meal as the percentage of extraction is increased. The values recorded along the horizontal axis are in terms of the entire wheat. In the instance of thiamine the greatest response in terms of the vitamin recorded in the flour appears when the extraction is increased from about 72 to about 78 per cent. This is in consequence of the high thiamine content of the 2nd clear and red dog flour, the

⁵⁸ *Ind. Eng. Chem., Anal. Ed.*, 14, 863 (1942).

addition of which to a straight grade might increase the percentage of recovered thiamine three or fourfold. In the instance of riboflavin the extraction must be increased above 75 per cent before the sharp increase in recovered vitamin appears, and even then it is not as great proportionately as with thiamine.

In addition to the spring wheats, Andrews *et al.*⁵³ determined the riboflavin content of a number of soft winter wheats, hard winter wheats, and four other cereals, with the results shown in Table 107. Since the spring wheats averaged 1.20 μg per g riboflavin, it appears that the riboflavin content of wheats of different market classes may be more constant than appeared in the instance of thiamine. It is also notable that

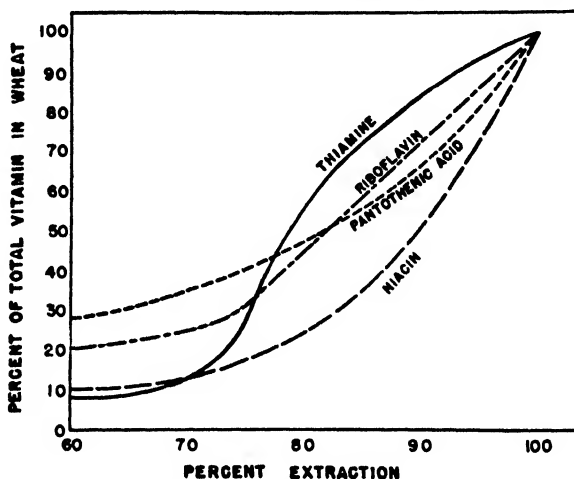


Fig. 17. Graphs showing the percentage of the total thiamine, riboflavin, and nicotinic acid in wheat that would be recovered at different levels of flour extraction, from the data of Andrews, Boyd and Gortner,⁵³ and of pantothenic acid from unpublished data by Elvehjem communicated by Andrews.

none of the other four cereals tested contained much more riboflavin than wheat, rye and corn being the highest but not to a major degree, while barley and oats have about the same content of riboflavin as wheat.

These analyses are in good agreement with those reported for whole wheat by Swaminathan,⁵⁴ who found it to contain 1.2 $\mu\text{g}/\text{g}$ of riboflavin.

The microbiological method of Snell and Strong was regarded by Barton-Wright⁵⁵ as superior to fluorometric methods, although different methods of extraction and manipulation prior to inoculation with *Lactobacillus helveticus* resulted in values ranging from 0.75 to 1.15 $\mu\text{g}/\text{g}$ of

⁵⁴ *Indian J. Med. Res.*, 30, 23 (1942).

⁵⁵ *Nature*, 149, 696 (1942).

Table 108. Riboflavin Content of Wheats, of Several Wheat Products and of Barley Oats, Rye and Barley, as Reported by Andrews, Boyd and Terry.⁵³

Description	No. of samples	—Riboflavin ($\mu\text{g/g}$)—	
		Average	Range
Soft winter wheats			
65 miscellaneous varieties	15	1.17	1.05-1.30
Kawvale	3	1.17	1.00-1.30
Hard winter wheats			
Turkey	7	1.22	1.15-1.30
Tenmarq	7	1.21	1.21-1.30
Chiefkan	6	1.09	1.00-1.20
Blackhull	7	1.17	1.10-1.25
Yellow corn	13	1.40	1.30-1.50
White corn	5	1.38	1.30-1.50
Barley	6	1.21	1.05-1.50
Oats	5	1.30	1.10-1.45
Rye	6	1.43	1.30-1.65

riboflavin in the instance of aliquots of a 75 per cent extraction straight-run flour. A method of extraction adopted by him involved hydrolyzing the material with 0.25N HCl for 15 minutes at 15 pounds pressure in an autoclave. The results of a number of determinations on different varieties of wheat, various fractions of the wheat grain, flours, and barley and rye were reported by Barton-Wright and appear here in Table 108.

Certain of these values appear to be higher than those reported by

Table 108. Riboflavin Content of Wheats, of Several Wheat Products and of Barley and Rye, as Reported by Barton-Wright.⁵⁵

Sample	Riboflavin content ($\mu\text{g/g}$)
Wheat, English	2.65
Wheat, Manitoba	2.9
Wheat, Australian	2.65
Wheat, Russian	3.7
Wheat, Testa	5.8
Aleurone layer	6.3
Wheat germ, commercial sample (1)	11.35
Wheat germ, commercial sample (2)	12.85
Wheat germ, commercial sample (3)	9.7
Flour A roll	0.4 - 0.5
Flour C roll	0.5 - 0.6
Flour, National straight run, 75% extraction	0.75- 1.25
Flour, National wheat meal, 85% extraction	2.0 - 2.1
Barley	2.3
Rye	2.65

Andrews, Boyd and Terry⁵³ for like material, and Barton-Wright suggests that Andrews, *et al.*, employed extracts that were too concentrated.

Flour beetles (*Tribolium confusum*) were used as test animals by Fraenkel and Blewett⁵⁶ in assaying flour and wheat germ for vitamin B. Apparently this insect does not need more vitamin B₁ than is present

⁵⁶ *Nature*, 147, 716 (1941).

in patent flour, since additions of thiamine to such flour did not alter its nutritive value for *T. confusum*. Additions of riboflavin (0.0155 mg/g) produced a marked effect, however, as evidenced by larval growth.

Since the application of the Snell and Strong microbiological method for the determination of riboflavin to flour enriched with riboflavin indicated the presence of much more of the vitamin than was added,

Table 109. Riboflavin Content of Cereal Products, as Determined by Different Methods by Scott, Randall and Hessel.⁵⁷

Material	Method—		
	Snell Strong ($\mu\text{g/g}$)	Modified Snell, Strong ($\mu\text{g/g}$)	Hodson, Norris Fluorometric ($\mu\text{g/g}$)
Patent flour			0.525
Patent flour + 3.675 $\mu\text{g/g}$ riboflavin	9.55	4.10	4.30
Bread from same flour	6.25	4.30	4.05
Whole-wheat flour	1.73	1.32	1.29
Red dog flour	4.45	2.65	2.80
Starch + 0.175 $\mu\text{g/g}$ riboflavin	0.21	0.175	0.175

Scott, Randall and Hessel⁵⁷ modified the method to include treatment of the autoclaved sample of cereal with takadiastase. The Snell and Strong method was then applied, as was also the Hodson and Norris⁵⁸ fluorometric method. From the data recorded in Table 106 it appears that the modified biological method obviated the stimulation of lactic-acid formation by the raw starch, which stimulation was destroyed by the enzymatic hydrolysis with takadiastase. The results were in good accord with those obtained by the fluorometric method of Hodson and Norris.

The Council on Foods of the American Medical Association³⁶ accepted an average value of 1.13 mg of riboflavin per pound of whole wheat. This is equivalent to 375 Sherman-Bourquin units per pound, or 0.25 mg/100 g, or 83 S-B units/100 g. For white flour the corresponding values were 0.18 mg/lb, or 60 S-B units/lb., or 0.04 mg/100g, or 0.13 S-B units/100 g.

While there was somewhat more riboflavin in the germ half of the wheat kernel than in the brush half, the concentrations being 0.58 and 0.32 mg/lb, respectively, according to Andrews,³⁸ the ratio of 1.81 to 1.00 was less great than in the instance of thiamine concentration, where the ratio was 5.80:1.00 in the instance of two halves.

When wheat kernels containing 1.0 $\mu\text{g/g}$ (dry matter basis) of riboflavin and 83 $\mu\text{g/g}$ of niacin were germinated for 4 days in water by Burkholder and McVeigh,⁵⁹ the riboflavin content increased to 2.4 $\mu\text{g/g}$,

⁵⁷ *J. Biol. Chem.*, **141**, 325 (1941).

⁵⁸ *Ibid.*, **131**, 621 (1939).

⁵⁹ *Proc. Nat. Acad. Sci. U. S.*, **28**, 440 (1943).

while the niacin decreased slightly to 72 $\mu\text{g/g}$. Although their data for germination of wheat in sand for 5 days are recorded only in graphs, the approximate values indicate increases in thiamine and niacin equivalent to about 50 per cent, a five-fold increase in riboflavin, and a 25 per cent increase in biotin. In a personal communication Burkholder advised that the treatment accorded the seed with CaClOCl solution seems to cause some loss in vitamin content. He transmitted some additional data resulting from the germination of wheat in peat moss at 25° for 5-6 days which follow:

Wheat	In micrograms per gram of dry matter						Folic acid
	Riboflavin	Niacin	Thiamine	Pantothenic acid	Pyridoxine	Biotin	
Dormant	1.3	62	7.0	7.6	2.6	0.17	28
Germinated	5.4	103	9.0	12.6	4.6	0.36	106

Knowledge respecting the nicotinic acid content of wheat, flour, and other cereal foods did not accumulate as rapidly as the information respecting thiamine and riboflavin. This has been the consequence of a lack until recently of convenient and wholly acceptable methods. Except for some work with dogs, animal assays were not put upon the quantitative basis that was true with thiamine assays, for example. As a result, many foods were originally merely listed as good, fair or poor sources of nicotinic acid. Recently several methods, both biological and chemical, have been proposed, and applied to a limited extent, and the resulting data are now beginning to appear in the literature. The situation is still confusing, however, since the conditions surrounding the extraction of the material to be assayed determines the apparent nicotinic acid to a disconcerting degree. As the data presented in the latter part of this section demonstrate, the presence or absence of alkalis in the extraction medium have a large bearing upon the results.

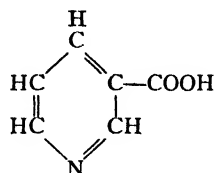
Table 110. A Summary of Data Respecting the Nicotinic Acid Content of Wheat and Wheat Products, as Reported by Quagliariello.¹¹

	mg/100 g	Reference
Wheat	5.33	Swaminathan, <i>Nature</i> , 141, 830 (1938).
Wheat, <i>durum</i>	3.95	Del Regno (in press)
Wheat, "tenero"	4.58	Del Regno (in press)
Flour, 80%	4.40	Del Regno (in press)
Flour, 00	3.81	Del Regno (in press)
Bran	5.10	Del Regno (in press)
Bran	5.00	Kringstad and Naess, <i>Z. physiol. Chem.</i> , 260, 108 (1939).
Germ	4.20	<i>Ibid.</i>

The early studies of the pellagra-preventive factor (P-P) of the vitamin B complex convinced Chick and Roscoe⁶⁰ that wheat germ was a poor source.

⁶⁰ *Biochem. J.*, 21, 698 (1927).

Surveys of two papers published prior to or during 1939, and of Del Regno's contemporary, but at that time unpublished data, were presented by Quagliariello ¹¹ and are recorded here in Table 110.



P—P, (Pellagra-preventive factor),
nicotinic acid, niacin

The nicotinic acid content of several cereals and cereal products was compiled from the literature by Bacharach.⁶¹ Some of these data not already recorded elsewhere are listed in Table 111.

A reaction of nicotinic acid, in consequence of the presence of a pyridine ring with cyanogen bromide and aniline, was employed by Swaminathan ⁶² in estimating the concentration of nicotinic acid in food. Hot aqueous extracts of the foodstuff were freed from protein derivatives by the addition of lead acetate, delead, the extract brought to pH 10 and

Table 111. Compilation from the Literature of the Nicotinic Acid of Wheat and Wheat products, as Reported by Bacharach.⁶¹

Material	Investigator	Citation	Nicotinic acid (mg/100 g)
Wheat germ	Kodicek	<i>Biochem. J.</i> , 34, 724 (1940)	2.7
	Waisman <i>et al.</i>	<i>J. Nutr.</i> , 19, 483 (1940)	4.0
	Kringstad and Naess	<i>Z. physiol. chem.</i> , 260, 108 (1939)	4.2
Wheat germ, extracted	Waisman <i>et al.</i>	<i>Loc. cit.</i>	6
Bran	Kringstad and Naess	<i>Loc. cit.</i>	5.0

decolorized with charcoal. The extract was then adjusted to pH 7 and made to volume. The aqueous cyanogen bromide solution was then added, followed by a saturated aqueous aniline solution, and the yellowish-green pigment that was produced was extracted by, and examined in pentanol.

When thus tested, whole wheat was found to contain 5.33 mg of nicotinic acid per 100 g (=24.2 mg per pound). White corn (maize) contained only 1.48 mg per 100 g (6.72 mg per lb), while dry milk solids less fat contained 10.53 mg per 100 g (47.8 mg per lb), and dried brewers yeast 62.50 mg per 100 g (283.75 mg per lb).

Wheat was found by Aykroyd and Swaminathan ⁶³ to contain 5

⁶¹ *Nutr. Abst. and Rev.*, 10, 459 (1941).

⁶² *Nature*, 141, 830 (1938).

⁶³ *Indian J. Med. Research*, 27, 667 (1940).

mg/100 g of nicotinic acid, flour 1 mg, and wheat germ 9.1 mg. These are equivalent to 50, 10 and 91 $\mu\text{g/g}$, respectively, and are in the same general range as were reported for Indian wheats and mill products by Shourie and Sundararajan,⁶⁴ namely, 48-50 $\mu\text{g/g}$ in wheat, 13 in white flour, and 16 in semolina. Corn (maize) was found by Aykroyd and Swaminathan⁶⁵ to contain less nicotinic acid than wheat, the average content reported by them being 1.3-1.6 mg/100 g, or 13-16 $\mu\text{g/g}$.

The nicotinic acid content of wheat germ, and of extracted wheat germ (commercial) was less than 4, and less than 6 mg per 100 g, respectively, when subjected to animal (dry) assays by Waismann, Michelsen, McKibbin, and Elvehjem.⁶⁵

Nicotinic acid in whole wheat and patent flour was determined by Melnick, Oser and Siegel.⁶⁶ The results were in fair agreement when de-

Table 112. Nicotinic Acid Content of Mill Products, as Determined and Reported Privately by E. B. Brown, Anheuser-Busch, Inc., June, 1931.

Material	Moisture (%)	Ash (%)	Nicotinic acid per pound (mg)
Wheat	11.53	1.52	6.0
Patent flour	13.68	0.38	1.6
Straight-grade flour	13.63	0.42	2.0
1st clear flour	13.47	0.67	4.1
2nd clear flour	13.30	0.88	7.3
Red dog	12.58	2.17	10.2
Shorts	13.00	3.94	13.7
Bran	13.98	5.89	20.4
Germ	11.30	3.54	24.1*
Defatted germ	11.30	3.54	18.3

* See text.

termined by (1) chemical, and (2) microbiological methods. Thus in whole-wheat flour they found 5.2-6.5 mg of nicotinic acid per 100 g of flour was determined by (1), and 4.5-7.0 mg by (2). In patent flour the quantities found by (1) were 1.3-1.9 mg and by (2) 1.0-2.0 mg.

The Bandier and Hald⁶⁷ method for the determination of nicotinic acid, involving the reaction with metol (*p*-methylaminophenol sulfate) and cyanogen bromide to give a yellow substance, was found by Bina, Thomas and Brown⁶⁸ to be unsatisfactory for application to flour and bread. They used takadiastase to hydrolyze the starch, and the Arnold, Schreffler and Lipsius⁶⁹ method could then be applied to such a digest. This method involves the use of *p*-aminoacetophenone instead of metol.

In a private communication Brown expanded the content of his paper

⁶⁴ *Ibid.*, 30, 61 (1942).

⁶⁵ *J. Nutr.*, 19, 483 (1940).

⁶⁶ *Ind. Eng. Chem., Anal. Ed.*, 13, 879 (1941).

⁶⁷ *Biochem. J.*, 33, 264 (1939).

⁶⁸ *Cereal Chem.*, 18, 661 (1941).

⁶⁹ *Ind. Eng. Chem., Anal. Ed.*, 13, 62 (1941).

to include the results of nicotinic acid determinations of a collection of mill products. Their data will be found in Table 112. The wheat germ contained interfering substances which gave an orange color. This was removed by defatting, however, and, accordingly, the second figure was regarded as more acceptable. The nicotinic acid content of the patent flour reported here is substantially less than was found by certain other investigators, and at this time it appears to be out of line with the general experience of the majority of workers in this field.

Unlike thiamine, the niacin in the wheat grain is not so largely concentrated in the germ half of the kernel. Thus the two halves, designated as germ half and brush half respectively, were found by Andrews³⁸ to contain almost exactly the same amount of niacin.

A microbiological (bacteriological) method was used by Teply and Elvehjem⁷⁰ in determining the nicotinic acid content of a representative series of hard wheat samples. The hard winter wheats ranged from 47 to 71 μg per g, and averaged 57, while the spring wheats ranged from 48 to 67 μg per g and averaged 55.

Using the microbiological method of Snell and Wright, the nicotinic acid content of a collection of 39 wheat samples was determined by Teply, Strong and Elvehjem.⁷¹ These wheats contained an average of 60.1 $\mu\text{g}/\text{g}$ of nicotinic acid, and ranged from 47 to 106 $\mu\text{g}/\text{g}$. About two-thirds of the samples contained between 50 and 60 $\mu\text{g}/\text{g}$. The average of the hard spring wheats was the highest of the several market classes, *i.e.*, 66.1 $\mu\text{g}/\text{g}$ of nicotinic acid, but it was also the most variable. In fact, there did not appear to be any well-defined correlation between this attribute of wheats and other properties or constituents.

On comparing four varieties of hard winter wheat grown in four locations in Kansas, the variety Chiefkan uniformly contained more nicotinic acid than Blackhull, Turkey, and Tenmarq, the average content being 66, 59, 58, and 57 $\mu\text{g}/\text{g}$ in the order named. Seven samples of wheat grown in Canada averaged highest in this constituent of any of the groups based upon locality where grown *i.e.*, 74 $\mu\text{g}/\text{g}$.

Products of milling were also assayed, with the following results:

	Nicotinic acid content ($\mu\text{g}/\text{g}$)
Patent flour	10
First clear flour	21
Second clear flour	57
Wheat germ	34
Average of 55 samples of wheat	59

As in other series of determinations the germ contained less nicotinic

⁷⁰ Private communication, March, 1941.

⁷¹ *J. Nutr.*, 24, 167 (1942).

acid than the wheat from which it was milled, which is not generally true of the other members of the vitamin B complex.

The type of extraction employed in determining the nicotinic acid content of cereal products was found by Andrews, Boyd and Gortner⁵⁸ to have substantial effect upon the results. Water and dilute acids yielded lower results than stronger acids and alkali. While there are

Table 113. Distribution of Nicotinic Acid in Products of Wheat Milling, as Determined by Microbiological Assay of Aqueous and Alkaline Extracts made by Andrews, Boyd and Gortner.⁵⁸

Mill Fraction	Nicotinic Acid		II/I	Total nicotinic acid in mill fraction (%)
	Aqueous (I) ($\mu\text{g/g}$)	Alkaline II ($\mu\text{g/g}$)		
Patent flour	6.6	12	1.83	10.2
First clear flour	13.4	26	1.94	3.2
Second clear flour	46	83	1.80	4.7
Red dog	74	120	1.62	7.6
Shorts	96	159	1.66	17.8
Bran	197	330	1.68	56.3
Germ	56	68	1.21	0.2
Wheat	38	70	1.82	100.0

several possible reasons for the differences observed, these investigators feel that their evidence supports the hypothesis that stronger acids and alkalies liberate active nicotinic acid compounds by hydrolysis of a less active or inactive precursor. If so, the choice of extraction medium would depend upon the nutritional availability of the substances in the food consumed. The ratio between the nicotinic acid disclosed by *Lactobacillus arabinosus* assay of aqueous and alkaline (1.5 per cent NaOH) digests of wheat and mill products is disclosed by the data in Table 113. The ratio is fairly constant in the instance of wheat and the several flours, but in wheat germ and the products rich in germ the difference between the two solvents is less great.

Their data also show that the distribution of nicotinic acid in the products of milling differs from that of either thiamine or riboflavin. This is shown graphically in Figure 18. Thus extending the percentage of extraction from 70 to 80 per cent results in a substantial increase in the recovery of thiamine and riboflavin, whereas the recovery of nicotinic acid under like circumstances is increased only nominally.

Snell and Wright's microbiological method was also used by Teply and Elvehjem⁷² in assaying another collection of hard and soft wheats for their nicotinic acid contents. The range was as follows:

Hard spring wheat 5.5 to 7.7 mg per 100 g
 Soft red winter wheat 5.2 to 6.7
 Hard red winter (1) 5.1
 Soft white spring (1) 5.3

⁷² Private communication, June, 1941.

Andrews and Gortner⁷³ determined nicotinic acid in a collection of patent flours by a chemical method involving some modifications of the aniline-cyanogendibromide method. The average was about 1.1 mg per 100/g and the range was relatively narrow.

When whole-wheat flour was autoclaved with 2N NaOH for 30 minutes by Cheldelin and Williams⁷⁴ the nicotinic acid content, as assayed by the Snell and Wright microbiological method, was equivalent to 65 $\mu\text{g/g}$. Autoclaving with water, followed by digestion with taka-diastase for 12 hours and then with papain for 12 hours, returned only 42 $\mu\text{g/g}$. The latter treatment applied to white flour disclosed 8.5 $\mu\text{g/g}$.

Alkaline or acid treatment was found by Hale, Davis and Baldwin⁷⁵ to be necessary for the complete utilization of nicotinic acid by the bacteria employed in the microbiological assay of Snell and Wright (I). Chemical assay values resulting from an application of a modification of the Kodicek procedure (II) were in good agreement with the assay values by the microbiological method as modified. In mg per cent, the nicotinic acid content of wheat by these two methods was (I) 7.6, and (II) 6.9, and that of wheat bran was (I) 26.3, and (II) 27.9, respectively.

Nicotinic acid, as well as riboflavin, was found to accelerate the growth rate of *Tribolium* in the experiments conducted by Barton-Wright.⁷⁶ Vitamin B₆ was also found to be essential for pupation of *Tribolium*, together with thiamine and some other growth factors.

While the results of her rat assays were not recorded in quantitative terms, Copping⁷⁷ observed that cereals are a good source of vitamin B₆. White wheat flour was inferior to whole wheat as a source of this vitamin, and it was concluded, therefore, that much of the vitamin B₆ of the wheat kernel is contained in the germ and integuments.

The pyridoxine or vitamin B₆ content of a collection of 33 wheat samples was determined by Teply and Elvehjem,⁷⁸ using the rat assay method of Conger and Elvehjem.⁷⁹ The range, stated as the hydrochloride, was from 3.2 to 6.3 μg per g. The average for the hard winter wheats was 4.5 μg per g, and for the hard spring wheats, 4.0 μg per g.

Pyridoxine was determined in a collection of wheat samples by Teply, Strong and Elvehjem,⁷¹ using the biological method of Conger and Elvehjem.⁸⁰ Blackhull wheat contained less pyridoxine than Chiefkan, Turkey, and Tenmarq winter wheats in three of the four localities in which it was grown in Kansas, containing 3.8 $\mu\text{g/g}$ as contrasted with 4.5,

⁷³ Private communication, June, 1941.

⁷⁴ *Ind. Eng. Chem., Anal. Ed.*, 14, 671 (1942).

⁷⁵ *Biol. Chem.*, 146, 553 (1942).

⁷⁶ *Nature*, 148, 565 (1941).

⁷⁷ *Biochem. J.*, 30, 849 (1936).

⁷⁸ Private communication, April, 1941.

⁷⁹ *J. Biol. Chem.*, 138, 555 (1941).

⁸⁰ *J. Biol. Chem.*, 138, 555 (1941).

5.4, and 5.0 $\mu\text{g/g}$ in the other three varieties respectively. There was no consistent correlation between pyridoxine content and other properties or constituents suggested by the data for a collection of 39 samples analyzed by these investigators. The average content was 4.51 $\mu\text{g/g}$, ranging from 3.2 to 6.3 $\mu\text{g/g}$. About two-thirds of these wheat samples contained between 4.0 and 5.0 $\mu\text{g/g}$ of pyridoxine.

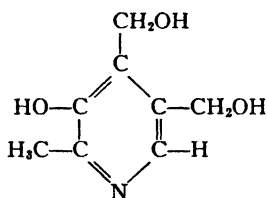
Patent flour contained nearly half as much pyridoxine as whole wheat, and wheat germ about twice as much, as shown by the following data.

	Pyridoxine content ($\mu\text{g/g}$)
Patent flour	2.2
First clear flour	3.9
Second clear flour	5.7
Wheat germ	9.6
Average of 55 wheat samples	4.6

Whole wheat was found by Atkin, Schultz, Williams and Frey⁸¹ to contain 4.7-4.8 $\mu\text{g/g}$ of pyridoxine, and patent flour contained 1.2 $\mu\text{g/g}$.

Microbiological assays involving the use of *Lactobacillus casei* E., as developed by Strong and Feeney, were applied to the determination of pantothenic acid in a collection of wheats studied by Teply and Elvehjem.⁸² With the medium used, the results were not entirely satisfactory, as evidenced by somewhat irregular curves. Since that time an important missing factor in the medium has been found to be asparagine. Upon the addition of this substance to the medium excellent results were obtained in a second group of assays.

The second series of wheats included 56 samples of all classes of bread wheats. These contained from 9.1 to 17.5 μg of pantothenic acid



Vitamin B₆, pyridoxine, 2-methyl-3-hydroxy-4, 5-di-hydroxy-methyl pyridine

per gram of wheat. The distribution in terms of pantothenic acid content was as follows: 9.1-11.0 $\mu\text{g/g}$, 19.6 per cent; 11.1-13.0 $\mu\text{g/g}$, 19.6 per cent; 13.1-15.1 $\mu\text{g/g}$, 32.2 per cent; 15.1-17.0 $\mu\text{g/g}$, 26.8 per cent, and over 17.0 $\mu\text{g/g}$, 1.8 per cent. Averages by classes, with the number of samples in parentheses, were as follows: Dark northern spring (11) 13.7 $\mu\text{g/g}$; hard white spring (4) 13.2 $\mu\text{g/g}$; soft white and white club (5) 11.2 $\mu\text{g/g}$; dark hard winter (27) 14.2 $\mu\text{g/g}$; soft red winter (9) 11.5 $\mu\text{g/g}$. In gen-

⁸¹ Ind. Eng. Chem., Anal. Ed., 15, 141 (1943).

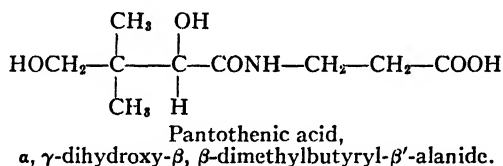
⁸² Private communication, November, 1940.

eral there was a tendency for the hard wheats to have a higher pantothenic acid content than the soft wheats.

Determination of pantothenic acid by their improved technique was extended by Teply and Elvehjem to include a series of products from a typical hard spring wheat mill. From their data, as tabulated below, it is evident that the refined patent grade of flour contains less of this member of the B complex than the lower grade flours or the germ, although the ratios are not as wide as in the instance of thiamine.

Mill Product	Pantothenic acid ($\mu\text{g/g}$)
Patent flour	5.7
1st clear flour	9.6
2nd clear flour	12.8
Germ	15.3

No well-defined effect of variety, or market class or locality where grown upon the pantothenic acid content of wheat is apparent in the observations of Teply, Strong and Elvehjem.⁷¹ In a collection of 39 wheat samples, the average pantothenic acid content, as determined by the method of Strong, Feeney and Earle,⁸³ was 12.64 $\mu\text{g/g}$, and the range was from 9.1 to 17.0 $\mu\text{g/g}$.



The studies of Elvehjem were extended to include determinations of pantothenic acid in flours and mill products representing progressively increasing extraction of wheat in the milling processes. His findings were presented graphically by J. S. Andrews³⁸ in a lecture before the Minnesota Institute of Cereal Chemistry in 1943, and are included in Figure 17. It is evident that there is less proportional change in pantothenic acid recovery in flour on extending the extraction than in the instance of any of the other members of the B complex that have been reported in such detail. The relative difference in this particular between a 72 and an 85 per cent extraction flour, for example, is much less than is the case with thiamine and riboflavin. Also the proportion of the pantothenic acid of wheat recovered in a straight-grade flour is substantially larger than in the other three vitamins here reported. This lends further emphasis to the conclusion that is steadily receiving added support, namely, that one cannot predict the recovery of one member of the B complex from the observations of another member of that complex.

⁸⁸ *Ind. Eng. Chem.*, 13, 566 (1941).

All cereals were found by Birch, György and Harris⁸⁴ to be rich in Vitamin B₆, maize being one of the richest. Thus one "rat-day dose" was found to be contained in 0.5 g of white maize, in 1.5 g whole wheat, 0.2 g of wheat germ, and 0.3 g of wheat bran. Thus it appears that this vitamin is not uniformly distributed in the wheat kernel, but is most prominent in the bran and germ. Quagliariello¹¹ apparently attempted to convert these observations of Birch *et al.* into terms of mg of B₆ per 100 g of material, with the following results: wheat 66, germ 500, bran 500. The exact basis for such a computation is not apparent, nor is it evident why he assigned the same value to germ and bran in view the recorded difference of Birch *et al.* (their Table III) in the quantity required to provide 1 "rat-day dose."

A method for the determination of choline, which involved extracting the material with absolute methanol, reducing the extract nearly to dryness, hydrolyzing the residue with boiling barium hydroxide, neutralizing with acetic acid, and, finally, precipitating the choline at 3° by the addition of reinecke salt was applied by Engel^{84a} to a number of plant and animal products. Among them were several wheat products, the choline content on the dry basis being reported in mg/g as follows:

Wheat	1.01	Germ	4.36
Bran	1.56	Defatted germ	4.53
Shorts	1.63	Flour	0.57

These data suggest that the germ is the richest in choline, endosperm contains the least, and the mill by-products which include the most germ likewise have the highest choline content. Since the germ tissue represents only about 2½ per cent of the kernel, a simple computation based upon the data for the flour and wheat indicate that the higher choline of the later is not attributable to that present in the germ, since, in that event, the wheat would contain only 0.68 mg/g. Accordingly, there must be a fairly high concentration in the pericarp and/or adherent fibrous layers or structures.

Biotin is included with the vitamins because of its apparent association with the respiratory function, and its reported identity with "Vitamin H."^{85,86,87} It was observed by Lampen, Bahler and Peterson⁸⁸ that the biotin in many natural products occurs primarily in a water-insoluble combination, and that substantial increases in the yield in extracts could be effected by acidulation of the extraction medium. While this was

⁸⁴ *Biochem. J.*, 29, 2880 (1935).

^{84a} *J. Nutr.*, 25, 441 (1943).

⁸⁵ *Science*, 91, 243 (1940).

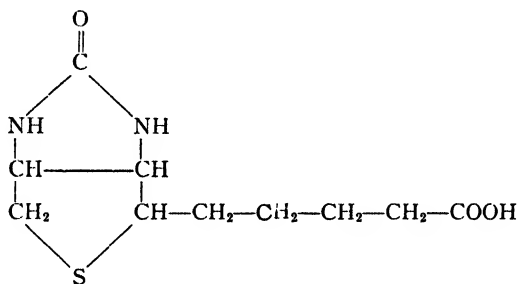
⁸⁶ *Ibid.*, 92, 62 (1940).

⁸⁷ *Ibid.*, 92, 809 (1940).

⁸⁸ *J. Nutr.*, 23, 11 (1942).

particularly true with yeast and meats, it was also evident, although to a lesser degree, with wheat and bran. Thus two samples of wheat showed a biotin content of 22 millimicrograms/g ($\text{m}\gamma/\text{g}$) and 113 $\text{m}\gamma/\text{g}$ respectively when extracted with water, and 67 and 70 $\text{m}\gamma/\text{g}$ when extracted with 4*N* H_2SO_4 . Wheat bran, when extracted with water showed 83 $\text{m}\gamma/\text{g}$ of biotin, but on extraction with 2*N* H_2SO_4 this was increased to 140 $\text{m}\gamma/\text{g}$. White flour contained much less biotin, the concentration being only 5.2 $\text{m}\gamma/\text{g}$ when extracted with 2*N* H_2SO_4 .

The structure of biotin has been the subject of considerable discussion. According to du Vigneaud *et al.*,⁸⁹ the following formula has been established.



Ascorbic acid or Vitamin C content of the normal or resting cereals is very low. Thus Pulkki and Puutula⁹⁰ recorded graphically a content of $10 \pm \text{mg}$ of ascorbic acid per kilogram dry weight of wheat before germination. A steady and substantial increase in this vitamin is shown graphically with elongation of the coleoptile, reaching 220 mg per kg ($=0.022$ per cent) when the coleoptile was 14 mm long. About 9/10 of the ascorbic acid was in the sprout, only 1/10 being found in the sprout-free residue of the grain. An increase of about sixfold in the ascorbic acid content of wheat during germination was observed by Muthanna and Ahmad.⁹¹

In whole wheat flour milled from freshly harvested wheat Rudra^{91a} found 5.3 mgm/gm of ascorbic acid as determined by 2:6-dichlorophenol indophenol titration. This was substantiated by biological assay. The symbol mgm/gm as used here must imply micrograms per gram, which would bring Rudra's findings into the same range as those reported by Pulkki and Puutula,⁹⁰ although somewhat lower.

The absorption spectrum of the Vitamin E fraction of wheat-germ oil was noted by Bowden and Moore.⁹² At the temperatures of liquid air the

⁸⁹ *J. Biol. Chem.*, **146**, 475 (1942).

⁹⁰ *Z. ges. Getreid-, Mühlen- u. Bäckereiw.*, **25**, 149 (1938).

⁹¹ *Current Sci. India*, **9**, 320 (1940).

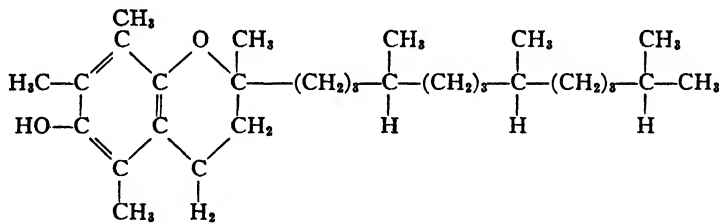
^{91a} *Nature*, **152**, No. 3848, p. 78 (1943).

⁹² *Nature*, **131**, 512 (1933).

absorption bands were sharper than at ordinary temperatures. Three well-defined peaks were evident in the blue with maxima at 4850, 4520 and 4260 Å. While these bands were in evidence at ordinary temperatures, and were detected in the original oil, they were increased fifty fold in the concentrate. In the ultraviolet there was a small band at 3370 Å, stronger absorption began at 3020 Å, followed by a small step-down at 2860, and a well-defined maximum at 2550 Å, followed by general absorption from 2400 Å down.

Morton and Edisbury⁹³ also studied the absorption spectrum of the unsaponifiable matter of wheat germ oil. The oil itself showed a maximum of 2720 Å, and the unsaponifiable gave maxima at 2800 and 2560 Å. On recrystallizing, a white solid showing ergosterol bands, and very distinct maxima at 2420 Å and 2530 Å was obtained. In general, Morton and Edisbury obtained indications of the presence of ergosterol in several preparations which they examined.

In an effort to concentrate or isolate the Vitamin E of wheat germ oil, Drummond, Singer and MacWalter⁹⁴ effected a considerable concentration from the unsaponifiable fraction, after removal of the greater part of the sterols, by fractional adsorption on aluminum oxide. The active fractions showed a well-marked absorption band with a maximum at 294 mμ, and a minimum at 267 mμ.



Vitamin E, alpha-tocopherol

The Furter and Meyer⁹⁵ method for the determination of the tocopherols (Vitamin E) involving oxidation to the red orthoquinone with nitric acid, improved to include chromatographic fractionation of the oxidation products on activated alumina, was applied by Binnington and Andrews⁹⁶ to bread wheat and its mill products. The method does not distinguish between alpha, beta and gamma tocopherol, but there is a basis for assuming that the alpha form is most prominently represented in such wheat products. Actually, the chemical determinations of to-

⁹³ *Ibid.*, 131, 618 (1933).

⁹⁴ *Biochem. J.*, 29, 456 (1935).

⁹⁵ *Helv. Chim. Acta.*, 22, 240 (1939).

⁹⁶ *Cereal Chem.*, 18, 678 (1939).

copherol, here reported in Table 114, were applied to the non-saponifiable matter of the oil extracted from these samples, and the resulting values computed to the basis of the entire original product.

Table 114. Mill Yield, Oil Content and Tocopherol Content and Distribution in Hard Wheat Milled Products, as Reported by Binnington and Andrews.⁹⁶

Sample	Mill yield (%)	Oil (%)	Tocopherol		
			In oil (%)	Mill product (mg/100 g)	Distribution (% of total)
Patent flour	60.3	0.83	0.003	0.03	2.0
First clear flour	9.4	1.78	.082	1.46	17.4
Second clear flour	4.1	4.16	.069	2.87	14.8
Red dog	2.7	5.83	.099	5.77	20.0
Shorts	9.3	4.41	.072	3.18	37.6
Bran	14.0	2.97	.012	0.30	6.3
Germ	0.1	8.90	.178	15.84	1.9
Whole wheat	—	1.54	.059	0.91	—

These data show that the tocopherol content of the oils from these several sources was not uniform, being lowest in the flour, and highest in the germ. The major part of the tocopherol was actually found in the red dog, shorts and clear flours, the patent flour being nearly devoid of it, and the bran being surprisingly low. The clear flours, red dog and shorts, while representing only about one-fourth of the mill products, contained about nine-tenths of the total tocopherol.

Assuming that the germ represents $2\frac{1}{2}$ per cent of the wheat kernel, and that its oil is as uniformly rich in tocopherol as that represented by the germ fraction recovered in this milling operation, it becomes apparent that much of the tocopherol found in certain of the mill products is due to the presence in them of germ fragments. Moreover, about 55 per cent of the total tocopherol in the grain should be found in the germ tissues. Since the endosperm is manifestly low, if not nearly devoid of tocopherol, it is apparent that about two-fifths of the total amount in the grain must be in certain of the pericarp or adjacent tissues surrounding the germ and endosperm.

Parallel results were not secured when observations were made upon the products of milling *durum* wheat. There was an indication that more of the Vitamin E was present in the endosperm tissues of this wheat.

By means of a selective adsorption technique or chromatogram Karrer and Salomon⁹⁷ recovered α -, β -, γ - and neo-tocopherol from the unsaponifiable fraction of wheat-germ oil. Using a potentiometric titration against gold (III) chloride, Karrer and Keller⁹⁸ found 0.103 per cent tocopherol in unsaponified wheat-germ oil.

Tocopherol content of products of milling increased substantially in

⁹⁷ *Helv. Chim. Acta.*, **21**, 514 (1938).

⁹⁸ *Ibid.*, **22**, 617 (1939).

progressing from the more to the less refined grades of flour in the studies reported by Engel.⁹⁹ This is evident from the data in Table 115. Wheat

Table 115. Tocopherol Content of Various Fractions of Milling, and Influence of Bleaching on the 0-70 per cent Flour, as Reported by Engel.⁹⁹

Degree of Milling (%)	Bleaching Agent	Tocopherol (mg/100 g)
0-70	None	1.7
70-80	"	2.7
80-82	"	4.8
82-90	"	5.9
90-100	"	5.9
1-100	"	2.6*
0-70	Unbleached	1.6
0-70	Benzoyl peroxide, weakly	1.1
0-70	Benzoyl peroxide, strongly	1.0
0-70	Nitrogen trichloride, weakly	1.2
0-70	Nitrogen trichloride, strongly	0.5

*Calculated

germ was found to contain 27 mg/100 g of tocopherol; but since it constituted only 2 per cent of the weight of the wheat, the tocopherol in the germ represented only about one-fifth of that in the entire kernel.

Bleaching flour with benzoyl peroxide and nitrogen trichloride reduced the tocopherol content, and the loss became greater as the concentration of the bleaching agent was increased.

⁹⁹ *Z. Vitaminforsch.*, 12, 220 (1942).

Author Index

A

Abbott, R. C., 75
 Abderhalden, E., 56, 57
 Adler, E., 299
 Adler, L., 257
 Ahlborg, K., 156
 Ahmad, B., 314
 Albizzati, C. M., 139, 236
 Alsberg, C. L., 42, 43, 46, 50, 51,
 80, 122, 142
 Alcock, A. W., 293
 Allen, E. W., 177
 Alpers, E., 186
 Alt, A., 58
 American Association Cereal
 Chemists, 257
 Amos, A. J., 184, 185, 188
 Ammann, L., 50
 Anderson, A., 233
 Anderson, J. A., 164, 269, 275,
 277
 Anderson, R. J., 200, 201, 222, 223
 Andrews, J. S., 224, 231, 285, 291,
 292, 296, 300, 301, 302, 303, 304,
 308, 309, 310, 312, 315
 Anonymous, 287
 Arany, S. A., 184, 260
 Armstrong, E. F., 160
 Arnold, A., 307
 Arpin, M., 121, 257
 Arragon, C., 256
 Arrhenius, S., 54
 Assn. Official Agr. Chemists, 257
 Astleford, 174
 Atkin, L., 282, 284, 295, 311
 Averil, H. F., 223, 224
 Aykroyd, W. R., 232, 299, 306

B

Badenhuizen, N. P. Jr., 141
 Bacharach, A. L., 306
 Bahler, G. P., 313
 Bailey, C. H., 32, 39, 62, 63, 70,
 76, 82, 108, 109, 110, 111, 112,
 119, 133, 134, 138, 140, 141, 142,
 149, 150, 183, 190, 191, 203, 207,
 209, 224, 255, 258, 263, 264, 266,
 268, 275, 276
 Baker, A. Z., 283, 289
 Baker, J. C., 146
 Baldwin, H. R., 310
 Balks, R., 241
 Ball, C. D., 186, 192
 Balland, A., 20, 23, 121, 124, 186,
 255, 256
 Balls, A. K., 104, 105, 194, 245
 Bandier, E., 307
 Barton-Wright, E. C., 192, 193,
 194, 303, 310
 Bartschat, Fr., 102
 Baston, G. H., 256
 Basu, U. P., 232
 Baughman, W. F., 186, 191
 Bear, R. S., 146
 Beath, O. A., 246
 Beccari, 11, 23
 Beckmann, C. O., 149, 159

Beeson, K. C., 214
 Bell, M., 282
 Belval, H., 170, 171, 172
 Benard, 121
 Benzon, B., 234
 Berzeller, L., 112, 181, 185, 208
 Bergmann, M., 160
 Berlin, A., 156
 Berliner, E., 122, 238
 Bernardini, L., 196, 197
 Bernfeld, P., 153
 Bertrand, G., 233, 234, 239, 243,
 244
 Berzelius, J. J., 14, 22
 Besley, H. J., 256
 Beythien, A., 195, 256
 Bibra, Freiherrn v., 16, 22, 119,
 176
 Bina, A. F., 307
 Bing, F. C., 232
 Binnington, D. S., 264, 266, 267,
 272, 315
 Birch, T. W., 283, 299, 313
 Birekner, V., 234, 257
 Bischoff, 20, 23, 67, 124
 Biswas, H. G., 288
 Bitte, B. V., 195
 Blaigman, S., 242
 Blewett, M., 303
 Blish, M. J., 32, 39, 43, 45, 48, 50,
 56, 61, 70, 75, 79, 87, 88, 89, 91,
 133, 138, 174
 Bloomfield, E. L., 230
 Bodalski, T., 223
 Bode, C. E., 215, 216
 Bonchardat, A., 15, 22
 Booher, L. E., 281
 Booth, R. G., 214, 215, 286, 288
 Bottazzi, F., 148
 Bouat, A., 245
 Boussingault, J. B., 14
 Boutwell, P. W., 221
 Bowden, F. P., 268, 269, 314
 Boyd, H. M., 300, 301, 302, 303,
 309
 Bracken, A. F., 138, 244
 Brazier, M. A. B., 55, 62, 82
 Breitwieser, K., 214
 Breteau, P., 256
 Bretschneider, R., 218
 Briggs, D. R., 70, 153
 Brockmann, H., 263
 Broda, B., 238
 Brown, E. B., 307
 Brown, H. T., 159
 Browning, B. L., 161
 Brownlee, W. E., 135
 Brückner, J., 203
 Bruere, P., 236
 Brunel, A., 132
 Bull, H. B., 55
 Burian, R., 129, 199
 Burk, N. F., 54
 Burkholder, P. R., 304
 Burr, G. O., 201
 Byers, H. G., 245, 247, 253

C

Cahours, A., 15, 22
 Cairns, A., 133, 135
 Caldwell, C. G., 158
 Cameron, C. A., 248
 Campbell, G. F., 128
 Cannon, H. J., 194
 Carrado, F., 236
 Carter, R. H., 214, 215
 Castoro, N., 132
 Cathcart, W. H., 290, 291
 Cerkez, S., 183
 Chamberlain, J. S., 35, 36, 49, 73,
 90
 Channon, H. J., 197
 Cheldelin, V. H., 310
 Chevat, H., 219
 Chiarulli, G., 197
 Chibnall, A. C., 61, 194
 Chick, H., 283, 305
 Chittenden, R. H., 21
 Cholnoky, L. V., 270
 Christie, A., 262
 Claydon, D. H. F., 179
 Clements, 287
 Coehn, A., 148
 Cohn, E. J., 54, 55, 57, 89
 Coleman, D. A., 259, 260, 262
 Colin, H., 170, 171, 172, 226
 Collison, R. C., 220
 Commaillie, A., 16, 22
 Conger, T. W., 310
 Conner, R. T., 289, 297, 299, 300
 Cook, W. H., 46, 57, 77, 80, 81,
 123
 Cooper, 119
 Copping, A. M., 283, 310
 Cormack, G. A., 184
 Council on Foods, Amer. Med.
 Assn., 208, 217, 238, 286, 304
 Coward, K. H., 263, 280
 Cramer, W., 281
 Crampton, C. A., 169
 Cranfield, H. T., 164
 Cross, R. J., 56, 78, 79, 84
 Croze, A. B., 263
 Csoska, F. A., 47, 51, 64, 70, 82,
 83, 84, 89, 102, 103, 116
 Cugnac, A. de, 172
 Cutler, G. H., 272

D

Dadswell, I. W., 208
 Dakin, H. D., 60, 80
 Dalby, G., 230, 236
 Damiens, A., 242
 Damodaran, M., 59, 61, 81
 Dangoumau, A., 184, 203
 Dartois, E., 221
 Davidson, J., 235
 Davis, C. F., 297
 Davis, G. K., 310
 De Caro, L., 283, 290
 Deininger, J., 257
 Del Regno, 305
 Dempwolf, O., 206
 Denham, H. G., 135

Deniges, 184
De Vaney, G. M., 250, 281, 298
Dill, D. B., 42, 50, 51, 120, 121,
122
Dingwall, A., 51, 53, 86
Di Stefano, F. de, 227
Dielatides, D., 33, 34
Dombrowsky, 256
Downs, D. E., 290, 291
Dox, A. W., 120
Drake, T. G. H., 289
Drummond, J. C., 204, 269, 283,
315
Dubaquie, 184
Dulac, J., 245
Düll, G., 176
Dumas, J. B., 15, 22
Dysterheft, G., 213, 214

E

Earle, A., 312
Eble, K., 218
Eckerson, S. H., 136
Edgar, J., 272, 289
Edisbury, J. R., 315
Einhof, H., 12, 22
Ellis, M. T., 200, 202
Elmer, R., 92, 133
Elvehjem, C. A., 229, 232, 233,
307, 308, 309, 310, 311, 312
Engel, Chr., 317
Engel, R. W., 313
Eppson, H. F., 246
Escue, R. B., 271
Eto, I., 44
Eto, J., 64
Euler, H. V., 270, 299
Eva, W. J., 164
Evans, J. W., 153
Ewing, J., 64, 89, 103

F

Fabris, 187
Fachinato, A., 256
Famiani, V., 283
Fanto, R., 39
Farrow, F. D., 158, 160
Feeney, R. E., 312
Felix, K., 61
Fellenberg, Th. V., 129
Felt, C., 231
Ferrari, C. G., 263, 264, 266, 276
Ferreira da Silva, A. J., 256
Feyte, A., 223, 227
Field, J., 162
Fifield, C. C., 258, 272
Fischer, E., 55
Fischer, G., 154
Fisher, E. A., 122
Fitz, L. A., 257
Fleurent, E., 21, 30, 34, 73
Flor, I. H., 230
Folin, O., 57, 58, 78, 82, 84
Forbes, E. B., 217
Ford, W. P., 242
Foreman, F. W., 59
Fosse, R., 132
Foster, C. A. M., 197
Fraenkel, G., 303
Franke, K. W., 248, 249, 252
Frankforter, E. B., 183
Frankforter, G. B., 185
Frankfurt, S., 132, 168, 169, 195
Fraps, G. S., 229, 230
Fredga, A., 251
Free, A. H., 232
Frederick, H., 283
Freeman, M. E., 178
Freilich, J., 139
Frey, C. N., 282, 284, 295, 311
Friedl, G., 50

Fudge, J. F., 229, 230
Furter, M., 315

G

Gasis, D., 117
Gatin-Gruzewska, Z., 147, 148
Geddes, W. F., 96, 98, 209, 264,
265, 266, 267, 271, 272, 273, 297,
298
Genevois, L., 173
Geoffroy, R., 34, 172, 177, 199, 225
Gericke, W. F., 115
Gersdorff, C. E. F., 57, 58, 60, 78,
91, 92, 103, 251
Gershon, A. I., 124, 127
Gerum, J., 120, 121
Gilbert, C. S., 246
Gill, A. H., 200
Girard, C., 256
Girardin, J., 121
Gisvold, O., 268
Glimm, E., 241
Gorelkina, T. M., 48, 151
Gortner, R. A., 32, 40, 45, 52, 57,
60, 64, 65, 66, 70, 74, 79, 93, 94,
96, 97, 99, 100, 102, 103, 176, 178
Gortner, W. A., 301, 302, 309, 310
Goswami H., 232
Gottenburg, M. J., 43
Goulden, C. H., 96, 98, 265, 271,
272
Graeve, P. de, 132
Graham, R. P., 163
Gralen, N., 54, 69, 70
Greaves, J. E., 38, 49, 50, 53, 211,
212, 217, 228, 233, 244
Green, J., 259
Greenburg, D. M., 55, 65
Grew, Emily, 32, 76, 140
Gröh, J., 50
Gubler, C. J., 244
Gudkow, 176
Guerrithault, B., 233
Guerrant, N. B., 220, 226
Guess, H. A., 30
Guest, H. H., 56, 59
Guillemet, R., 173, 243
Günsberg, R., 16
Günther, T., 256
Gustafson, C. B., 186
György, P., 209, 313

H

Haas, P., 176
Haerdth, H., 149
Hald, J., 307
Hale, E. B., 310
Hale, W. S., 104, 105, 194, 245
Halton, P., 50, 87, 88, 122
Hanke, U., 229
Hanna, W. F., 268
Hanson, E. A., 142, 143
Harding, E. F., 183, 185, 213, 214
Harris, 287
Harris, I. F., 50, 129
Harris, G. H., 159
Harris, L. J., 280, 283, 299, 313
Harris, R. H., 63, 93, 99, 100, 123
Harris, T. H., 104, 105
Harcourt, R., 87, 73, 174, 175
Hardy, W. B., 122, 124
Hart, E. B., 218, 232
Hartmann, B. G., 162
Hartsing, T. F., 215, 216
Hartzler, E. R., 281
Hathaway, J. C., 240, 241
Haugaard, G., 47, 51, 52, 54, 57,
60, 70
Hausmann, W., 55
Haworth, W. N., 155, 160
Hayashi, T., 48, 64, 89

Hays, J. G., 228
Hegudus, I., 218
Heiduschke, A., 257
Heinrich, R., 205
Heizer, H. K., 215, 216
Hendry, J. L., 54, 55, 57, 89
Herd, C. W., 40, 96, 122, 161, 184,
185, 188, 263, 274
Herrington, B. L., 39, 40, 74, 138
Herzner, R., 92
Hess, K., 158
Hess, W. C., 83, 84, 85, 139
Hessel, F. H., 304
Heyl, F. W., 126
Hiestand, O., 196
Higginbotham, R. S., 158, 160
Hiditch, T. P., 190
Hill, A. V., 176
Hillick, F., 162
Hilmoe, R. J., 252
Hinton, J. J. C., 292, 293
Hirsch, P., 57, 66, 83, 104
Hirst, C. T., 211, 212, 217
Hirst, E. L., 147, 148, 157
Hixon, R. M., 158
Hoagland, R., 38
Hodges, M. A., 212
Hodson, A. Z., 299, 304
Hoffman, Chas., 230, 236
Hoffman, W. F., 57, 64, 65, 66,
79, 93, 94, 96, 97, 100, 102, 103,
176, 178
Holl, H., 66
Hopkins, C. Y., 162, 163
Horn, M. J., 47, 51, 84, 251
Houk, A. E. H., 270
Howe, M., 139, 191, 192, 211, 240,
242
Hudson, C. S., 158
Hunt, C. H., 280
Hunt, M. J., 283
Hurd-Karrer, A. M., 245, 247
Husemann, E., 155, 158, 160
Hutchinson, W. S., 207

I

Ibanez, R. P., 242
Ichiba, A., 202
Iddles, H. A., 149, 151, 159
Imas, S., 235
Immer, F. R., 113, 185, 209
Irvine, J. C., 155
Itallie, T. B. van, 144, 145

J

Jaaback, G., 61
Jackson, H. M., 283
Jackson, S. H., 259, 296
Jacobs, B. R., 122, 183
Jaecle, H., 195
Jago, W., 164
Jamieson, G. S., 186, 191
Janicki, J., 134
Jansma, F., 233
Jarosz, W., 227
Javillier, M., 33, 34, 226, 235, 237
Jodidi, S. L., 132
Johannsen, W., 20, 23
Johannson, H., 283, 289
Johnson, A. H., 47, 51, 52, 54, 57,
60, 70, 136, 208, 217, 259
Johnson, L. R., 253
Johnson, S. W., 72
Johnson, T. B., 129
Jones, C. R., 214, 215
Jones, D. B., 51, 57, 58, 59, 60,
64, 70, 78, 80, 82, 84, 89, 91, 92,
103, 104, 107, 261
Jong, H. L. Bungenberg de, 124,
125
Jorgenson, H., 262

K

Kalning, H., 257
Kaphammer, J., 60, 80
Karrer, P., 316
Katz, J. R., 142, 143, 144, 145
Keding, E., 202
Keller, H., 318
Kemp, I., 65
Kennedy, M. H., 248, 250
Kent-Jones, D. W., 122, 161, 242, 263, 274

Kent, N. L., 237
Kepner, B. H., 121
Keresztesy, J. C., 150
King, C. G., 223, 224
Kirchoff, G. S. C., 154
Kjeldahl, J., 36, 49, 50
Klaar, W. J., 125
Klemm, M., 213
Knehe, E., 160
Kniaginicev, M. I., 48
Kniaginichev, M. I., 151, 208
Knowles, F., 219, 220
Knyaginichev, M. I., 115
Kocsis, E. A., 218
Kodicek, E., 306
Koets, P., 150
Kolachov, P., 164
Komatsu, S., 61
Kondo, K., 48, 52, 64, 86, 89
König, J., 23, 32, 102, 108, 133
Koopmann, J., 122
Kosmin, N., 31
Kossel, A., 50, 56
Kosutany, T., 31, 67, 106, 196
Kramer, M. M., 281
Kratky, O., 157
Kreis, H., 256
Krejci, L., 54, 69, 102
Kretovic, W., 41
Kretowitch, W., 77, 168
Kretowitch, W., 40
Kringstad, H., 299, 305, 306
Krug, W. H., 167
Kuhl, H., 68, 277
Kuhlmann, A. G., 67, 126
Kuhn, R., 263, 299
Kütcher, F., 50, 56, 78

L

Labat, 184
Labour, G., 34
Ladd, E. F., 37, 73
La Forge, F. B., 129
Lake, G. C., 118
Lakin, H. W., 245, 253
Lamm, O., 54, 159
Lampen, J. O., 313
Landauer, M., 103
Landis, Q., 159
Larmour, R. K., 56, 59, 79, 80, 84, 85, 293
Laufer, S., 297
Leach, A. E., 232
Le Clerc, J. A., 122, 131
Lehmann, K. B., 232
Lehrmann, L., 152
Leichsenring, J. M., 230
Leong, P. C., 281, 283
Lesetre, E. N., 157
Leulier, A., 219
Levene, P. A., 129
Levi, I., 164
Levine, M. N., 297, 298
Levine, V. E., 248
Levy, G., 234
Liebig, J. v., 14, 15, 22, 168
Lifschitz, D. B., 179
Lindet, L., 50
Lindow, C. W., 233, 235
Ling, A. R., 147

Lintner, C. J., 176
Lippmann, E. von, 160
Lipsius, S. T., 307
Looney, J. M., 57, 58, 78, 82, 84
Lounsberry, W. C., 131
Lüers, H., 48, 103, 257
Lunde, G., 299
Lung, K. H., 158
Luttringhaus, A., 203
Lyman, J. F., 294

M

MacLeod, G., 232
MacWalter, R. J., 204, 269, 315
Maiden, A. M., 242
Malmberg, M., 270
Maltha, P. R. A., 296
Mangels, C. E., 100, 117, 121, 138, 142, 143, 189, 202
Manget, Ch., 73
Mark, H., 157
Markley, K. S., 132
Markley, M. C., 258, 268, 273, 275
Marotta, D., 122
Martin, A. L., 247
Martin, J. J., 117
Martin, S. H. C., 21, 23
Martin, W. M., 189, 190
Mason, G., 217
Masters, M., 245
Mathewson, W. E., 50
Maume, L., 245
May, C. E., 57, 58, 78, 82
Mayer, A., 159
Mayer, F., 132, 199
Mayer, K., 151
McCalla, A. G., 54, 68, 69, 70, 199, 237
McCance, R. A., 212, 230, 245
McClendon, J. F., 240, 241
McHarue, J. S., 212, 235
McKibbin, J. M., 307
McVeigh, I., 304
Melnick, D., 307
Melo, M. S., 260
Mendel, L. B., 282
Metzger, 120, 121
Meyer, A., 146
Meyer, K., 157
Meyer, K. H., 152
Meyer, R. E., 315
Michaelis, L., 64
Mickelsen, O., 307
Miller, H., 289
Miller, J. T., 245, 247
Minaeff, M., 149
Mituda, H., 86
Moeller, O., 58, 59, 78, 80
Mokragatz, M., 233
Monier-Williams, G. W., 261
Moore, M. G., 200
Moore, T., 268, 269, 314
Moran, T., 214, 215, 227, 228
Morgan, A. F., 283
Morishimo, K., 21
Morris, V. H., 215, 216
Morton, R. A., 315
Mottram, J. C., 281
Moxon, A. L., 246, 247, 248, 249, 252
Mullen, J. W., 154
Müller, R., 278
Munsell, H. E., 250, 261, 264, 298, 299
Munsey, V. E., 269
Muntoni, F., 227
Munts, M. A., 169
Murphy, J. C., 64, 108
Muthanna, M., 814
Murthy, G. N., 298

Myers, D. R., 157
Mygind, H. G., 61
Myrbäck, K., 156

N

Nabenhauer, F. P., 201
Naegeli, C., 146
Naess, T., 305, 306
Nakamura, N., 202
Nanji, D. R., 147, 181
Naszalyi, N., 209
Near, C., 183, 197, 198, 210, 217, 219, 237
Nedokutschajew, N., 136
Neglia, F. J., 83, 84, 85
Negri, G. de, 182, 187
Nelson, D. H., 212
Nelson, E. M., 251
Nelson, J. M., 152
Neurath, H., 55
Newcomb, C., 235
Newman, L. H., 289
Nikolaieff, V. A., 141
Nordgren, R., 285, 291, 292
Norman, A. G., 181
Norris, F. W., 177, 180
Norris, L. C., 299, 304
Norton, F. A., 31, 120
Notkina, L. G., 179
Notthohn, F. E., 132, 199
Nottin, P., 221

O

Obermeyer, H. G., 146, 148
O'Brien, M., 21, 23, 67
Oeholm, L., 159
Olsen, A., 299
Olsen, A. G., 133, 135
Olson, G. A., 38, 91, 120, 137
Olson, O. E., 246, 247, 252
Osborne, T. B., 23 ff, 30, 35, 44, 50, 54, 56, 59, 72, 78, 84, 90, 118, 119, 128, 129
Oser, B. L., 307
O'Sullivan, C., 167, 169
Otis, S. G., 232

P

Pacsu, E., 154
Padoa, M. L., 123
Painter, E. P., 248, 249, 250, 252
Palmer, L. S., 268
Pap, L., 242
Pascoe, T. A., 99
Patten, A. J., 218
Pavloff, M., 173
Pearsall, W. H., 64, 89, 103
Pecaud, M. T., 257
Pedersen, K. O., 54
Pelshenke, P., 185, 242
Percival, E. G. V., 160
Peterson, W. H., 212, 229, 232, 233, 235, 313
Pieri, C., 272
Pietz, J., 179
Pinckney, A. J., 48, 50, 87
Planchon, 256
Plant, M. M. T., 147, 157
Platenius, H., 75
Plimmer, R. H. A., 75
Plucker, 187
Polson, A., 54
Popowa, A. I., 31
Posternak, M. S., 218
Posternak, S., 225
Potter, V. R., 249
Power, F. B., 201
Pratolongo, U., 82
Preece, I. A., 177, 180
Prentiss, A. M., 54, 55, 57, 89
Pringle, W. J. S., 228

Prior, E., 256
 Proskuryakov, N., 217
 Pulkki, L. H., 314
 Putnam, F. W., 70
 Puutula, K., 314

Q

Quagliariello, G., 226, 227, 282,
 299, 305, 306, 313

R

Rakowski, A. V., 165, 166
 Rammstedt, O., 32, 256
 Randall, F. E., 304
 Ranganathan, S., 232
 Rask, O. S., 142, 183, 240
 Rather, J. B., 218, 221, 222
 Reichert, E. T., 142
 Reindl, H., 61
 Reinitzer, 200
 Retznikoff, P., 230
 Riazanceva, E., 41
 Rice, W., 175
 Rich, C. E., 100, 101, 288, 289
 Richardson, C., 169
 Richardson, W. A., 158, 160
 Rideal, E. K., 65
 Rintelen, P., 23, 32
 Ritter, F., 200
 Ritthausen, H., 17, 18, 19, 20, 21,
 22, 119, 176, 177, 199
 Rivot, M., 119
 Robertson, T. B., 53
 Robinson, W. O., 248, 253, 254
 Rodewald, H., 158
 Rona, P., 64
 Ronzin, N., 149
 Roscoe, M. H., 283, 299, 305
 Rose, E. R., 57, 58, 78, 82
 Rose, M. S., 230, 232
 Rose, R. C., 68, 77, 81, 123
 Rosedale, J. L., 75
 Rosenblatt, M., 239
 Rotsch, A., 179
 Rousseaux, E., 217
 Roy, G. K., 299
 Ruter, R., 238

S

Saiki, T. H., 232
 Salatan, L., 297
 Sallans, H. R., 59, 84, 85
 Salomon, H., 316
 Salway, A. H., 201
 Salzmann, R., 150
 Samec, M., 148, 149, 150, 151,
 159, 165
 Samuel, L. W., 117, 135
 Samuely, F., 56, 57
 Sandals, K. M., 246
 Sande-Bakhuysen, H. L., 143
 Sandstedt, R. M., 32, 43, 45, 61,
 70, 75, 79, 89, 174
 Sankaran, G., 235
 Sarata, U., 64
 Sarasin, J., 132
 Saussure, T. de, 14, 22
 Scheele, K., 162
 Schell, C., 173
 Scherer, J., 15, 22
 Scherts, F. M., 263, 266
 Scheunert, A., 283, 284, 299
 Schiebllich, M., 253, 299
 Schindler, J., 256
 Schleimer, A., 38
 Schlötzer, A., 299
 Schmalz, F. D., 139
 Schmidt, C. L. A., 55
 Schmidt, E., 185
 Schmorl, K., 126, 213
 Schoch, T. J., 162

Schofield, R. K., 117
 Schreffler, C. B., 307
 Schrumpf-Pierron, P., 209, 210
 Schryver, S. B., 179, 180
 Schulerud, A., 257, 259, 276, 277
 Schultz, A. S., 284, 295, 311
 Schultz, N. P., 141
 Schulze, E., 132, 168, 169, 176,
 177, 195, 197
 Schweitzer, T. R., 230, 236
 Scott, M. L., 304
 Scott, S. G., 136, 217
 Scott Blair, G. W., 135
 Seairight, W. V., 246
 Secchi, G., 260
 Seck, W. von, 154
 Shackleton, L., 230
 Sharp, P. F., 32, 39, 40, 74, 92,
 133, 134
 Shell, C., 243
 Sherman, H. C., 146, 230, 232
 Sherman, W. C., 232
 Sherwood, R. C., 99, 134, 209,
 291, 292
 Shetlar, M. R., 294
 Shimizu, G., 241
 Shirahama, K., 241
 Shourie, K. L., 290, 307
 Shriner, R. L., 201
 Shutt, F. T., 32, 133, 168
 Sibbitt, L. D., 266
 Siegel, L., 307
 Silberstein, L., 243, 244
 Simerl, L. E., 161
 Simpson, A. G., 273, 274
 Sinclair, A. T., 199
 Sinclair, W. B., 45, 52, 60, 94,
 96, 97, 100, 102
 Singer, E., 204, 289, 315
 Sirot, M., 217
 Sjoström, O. A., 142
 Skinner, J. T., 235
 Smirnov, V., 123
 Smith, E. E., 21
 Smith, M. I., 251, 252
 Smith, T. C., 232
 Smolenski, K., 196
 Snyder, H., 30, 35, 36, 37, 73
 Sorenson, S. P. L., 52
 Spaeth, E., 187
 Spalding, R. C., 242
 Spencer, E. Y., 69
 Spielman, M., 203
 Spörer, H., 60, 80
 Sprague, H. B., 263
 Squire, M., 283
 Staker, E. V., 96, 97
 Stamborg, O. E., 140, 141, 149,
 150
 Stark, W. H., 164
 Staudinger, S. H., 155, 158, 160
 Steenbock, H., 280
 Steiger, E., 176, 195
 Stoa, T. E., 138
 Stockelbach, L. S., 62, 70, 82
 Stoll, A., 268
 Stone, W. E., 167, 176
 Stoppel, F., 57, 83, 104
 Straub, G. H., 289
 Straub, G. J., 299, 300
 Strohecker, R., 214
 Strong, F. M., 308, 310, 312
 Stuart, L. S., 105
 Stutzer, A., 131, 133, 137
 Sullivan, B., 139, 183, 190, 191,
 192, 197, 198, 203, 210, 211, 217,
 219, 237, 240, 242
 Sullivan, M. X., 83, 84, 85, 189
 Sundararajan, A. R., 290, 307
 Svedburg, T., 54, 69, 102
 Svensson, G., 162

Swain, R. E., 56, 78, 79, 84
 Swaminathan, M., 305, 308
 Swanson, C. O., 136, 137, 216, 257
 Synge, R. L. M., 61

T

Taddei, G., 12, 22
 Tague, E. L., 44, 89, 136, 137
 Tanret, C., 146, 170, 171
 Taylor, A. E., 286
 Taylor, T. C., 149, 150, 151, 152,
 159
 Teller, G. L., 35, 60, 77, 90, 100,
 109, 131, 132, 158, 177, 261
 Teller, W. K., 60, 77, 100
 Temerin, S., 217
 Teply, L. J., 308, 309, 310, 311, 312
 Terry, E. E., 300, 301, 303
 Thatcher, R. W., 37, 116, 137
 Thomas, A., 130
 Thomas, A. W., 158
 Thomas, E. M., 180
 Thomas, J. M., 307
 Thomas, P. E., 132
 Thorwaldson, T., 253
 Tillmans, J., 57, 58, 83, 104
 Tisdall, F. F., 289, 290
 Tollens, B., 176, 177
 Töpfer, A., 184
 Toscani, V., 230
 Town, B. W., 59
 Trelease, S. F., 247
 Troensegaard, N., 51, 61
 Tufts, C. G., 200

U

Utt, C. A. A., 240

V

Vahlteich, E. M., 230, 232
 Van Slyke, D. D., 55, 56
 Vaubel, R., 214
 Vedrödi, V., 232, 233
 Vericillo, A., 122
 Victorow, C., 148
 Vigneaud, V. du, 314
 Voorhees, C. G., 119
 Voss, J., 262

W

Wadham, 287
 Waisman, H. A., 306, 307
 Wakeham, G., 206
 Walde, A. W., 189
 Walden, P., 159
 Waldschmidt-Leitz, E., 151
 Walton, R. P., 151
 Wanklyn, 119
 Ward, Alan H., 292, 293
 Wastl, H., 112, 208
 Wastl, W., 181, 185
 Watkin, J. E., 219, 220
 Webster, J. E., 224, 233
 Weller, G., 139
 Wells, H. G., 118
 Wertheim, W., 153
 Wesener, J. A., 261
 Westfall, B. B., 251, 252
 Weyl, T., 20, 23, 67, 124
 Wheeler, L. H., 129
 Whitcomb, W. O., 189, 190, 208
 White, H. L., 255, 256
 White, S. N., 252
 Whitehead, E. I., 252
 Whiteside, A. G. O., 264, 265,
 271, 272, 289, 295, 296
 Wiazownicka, H., 64, 226
 Widdowson, E. M., 212, 227
 Widtsoe, J. A., 177
 Wiles, H. O., 53
 Wiley, H. W., 167

Wilkinson, M. D., 147, 157
Willard, J. T., 257
Williams, K. T., 245
Williams, R. J., 310
Williams, W. L., 311
Willkie, H. F., 164
Willstätter, R., 268
Wilson, H. E. C., 299
Wilson, R., 58
Windaus, A., 203
Winterstein, E., 196
Winton, A. L., 261, 292

Winton, K. B., 292
Wodehouse, R. P., 118
Wolff, E. T. von, 205
Wolfrom, M. L., 157
Wood, T. B., 32, 122, 124
Woodford, E. K., 237
Woodman, H. E., 50, 51, 65, 87
Woodruff, Sybil, 148
Word, A. H., 206
Worzella, W. W., 272
Wrampelmeyer, E., 195
Wright, M. D., 283, 289

Y
Yamada, T., 52
Young, 287
Young, S. M., 228
Yukitomo, T., 61

Z
Zaharia, A., 112, 181, 185, 208
Zalar, J. F., 238
Zechmeister, L., 270, 271
Zeleny, L., 259, 260
Zinn, J., 32
Zunini, E., 214, 226

Subject Index

- Absorption bands, of lipoids, 185
 - of sterols of flour, 204
- Absorption spectra, of gliadin preparations, 52
 - of vitamin E, 314, 315
- Acetic acid, as gliadin solvent, 43
 - as prolamins solvent, 26
 - as protein precipitant, 133
 - effect on spec. rot. power of glutenin, 87
 - spec. rot. power of gliadin in, 36
- Acetone extracts, 189
- Acetyl number of fats, 190, 191
- Acid binding, gliadin, 65, 66
 - proteins, 117
- Acid digestion, in niacin determination, 309, 310
- Acid dispersion of protein, 117
- Acid, effect on biotin determination, 313, 314
- Acid number of fats, 188
- Acid value, bran and flour fats, 192
- Acidity, 255 ff
- Acidity and viscosity of gliadin solutions, 48
- Adenine, in embryo nucleic acid, 128
- Adsorption of amyloses, 154
- Albumin, 90 ff
 - (Dumas and Cahours), 14, 15, 22
 - extracted from wheat species, 97
 - from bran, 91
 - in wheat, flour and bran, 100
 - nitrogen factor, 107
 - (Osborne), 24, 28
 - (Ritthausen), 20, 22
- Albumin "A" (Hoffman and Gortner), 93
 - "B" (Hoffman and Gortner), 93
- Albuminate (Martin), 21
- Albuminose (Bonchardat), 15, 22
- Albumose (Martin), 21
- Aleurone cells, identification, 238
- Alcohol-soluble proteins, 12, 22, 30
- Alkali binding, gliadin, 65, 66
- Alkali digestion in niacin determinations, 309, 310
 - "Alkali disease," 245
- Alkali dispersion of gluten, 80, 81
- Alkali, effect on proteins, 47
 - effect on spec. rot. power of glutenin, 87
- Allantoin in wheat, 132
- Alumina, in adsorption of tocopherols, 315
- Aluminum, in mill products, 210, 234
- Aluminum hydroxide, as protein precipitant, 133
- Amer. Med. Ass'n., Council on Foods, Ca content of wheat and flour, 238
 - Iron content of wheat, 217
 - P content of wheat and flour, 217
 - Riboflavin values, 304
 - Thiamine value, 288
- p-Amino acetophenone in niacin determination, 307
- Amino acids, changes during maturation, 133, 137
 - from crude gluten, 123
 - increase with time of tempering, 137
 - in wheat protein, 28, 116
 - in wheat and flour, 131 ff
 - of gliadin, 56, 57
 - role in acidity, 260
- Amino compounds, and acidity, 257
- Ammonium sulfate fertilizer, and thiamine in wheat, 280
- Ammonium thiocyanate as gliadin solvent, 43
- Amylan, 169, 170
- Amylohemiacellulose, 147
- Amylopectin, 145, 146, 147, 148, 149, 150, 153, 154
 - silica in, 214
- Amyloses, 146 ff
 - α -Amylose, 146, 151, 154
 - β -Amylose, 146, 148, 151, 154
- Amyrin, in germ, 269
- β -Amyrin, 204
- Anaphylaxis reactions with proteins, 117, 118
- Anhydro-oxy-methylene-diphosphoric acid, 218
- Araban, in flour, 173, 177
- Arabinose, from pentosans, 176
- Arginine, changes during maturation, 136
 - in wheat, 116
- Arginine nitrogen in gliadin, 59
- Arsenic, in flour, 215
 - in wheat, 215
- Artolin (Morishimo), 21
- Asparagine, changes during maturation, 136
 - in wheat germ, 132
- Aspartic acid, in gliadin, 59
- Ash, correlation between flour ash and sol. protein, 101
 - correlation with P content, 217
 - correlation with thiamine content, 285, 288
 - correlation of wheat and flour, 209
 - effect of weathering of wheat, 208
 - in Canadian wheats, 209
 - in mill products, 210
 - in Rumanian wheats, 209
 - of wheat, composition, 237
 - phosphorus content, 216 ff
 - relation to amino acids in flour, 136
 - relation to soil moisture, 208
- Astragalus bisulcatus, 246, 247
- Asymmetries of proteins, 55
- Ata, 288
- Australian flour, thiamine content, 287
- Aust. Nat'l. Health & Med. Council, thiamine studies, 287
- Australian wheat, riboflavin content, 303
 - thiamine content, 286, 287
- Auramin-Eosin color standard, 276
- Azobenzene as pigment standard, 263
- Bactericidal properties of crystalline protein, 105
- Bandier and Hald method for niacin determination, 307
- Barium
 - in flour, 215
 - in wheat, 215
- Basic nitrogen, in gliadin, 59
- Benzoyl peroxide, effect on tocopherol in flour, 317
- Benzyl alcohol as prolamins solvent, 26
- Betain in wheat germ and bran, 132
- Biotin, 313 ff
 - structural formula, 314
- Bleaching, effect on tocopherol in flour, 317
- Boron, in flour, 215
 - in wheat, 215
- Bran, albumin and globulin in, 91, 100
 - albumin content, 234
 - betain in, 132
 - calcium content, 238
 - chlorine content, 240
 - choline content, 313
 - cobalt content, 234

- Bran—(Continued)
 copper content, 233, 234
 fat constants, 192
 fat content, 185
 gum from, 176
 hemicelluloses, 177, 180
 inorganic P in, 220, 221
 iodine in, 241
 iron in, 230, 231
 isoelectric point of proteins, 103
 lead content, 237
 lecithin content, 195
 lecithin P, 217
 lipids, 197, 198
 lipid phosphorus in, 219, 220
 magnesium content, 237
 manganese content, 235
 minerals of, 206, 210, 212
 niacin content, 305, 306, 307, 309, 310
 nickel content, 234
 nuclein P, 217
 phosphorus compounds, 224
 phytin, in, 221, 228
 phytin P, 217, 228
 phytosterol in, 219, 220
 pigments, 274, 276, 278
 prolamin, 58, 60
 proteins from, 91
 riboflavin content, 299, 300, 301
 silver content, 237
 starch content, 184
 sulfur/protein ratio, 242, 244
 thiamine content, 282, 287, 289, 291, 292, 293, 294
 tin content, 237
 tocopherol content, 316
 unsaponifiable matter, 201
 vitamin B content, 281
 zinc content, 234
 Branched chain structure of starch, 155, 156
 Bread, manganese content, 235
 minerals in serving, 212
 Bromine, in wheat and flour, 215, 242
 Bromine No. of fats, 188
 Butanol as solvent for carotinoids, 266, 273

 Calcium, distribution in mill products, 238
 flour, %, 238
 flour grades, 216
 in Egyptian wheats, 209
 in flours, 212
 in mill products, 206, 209, 210
 in mill streams, 214, 238
 in Utah wheats, 211
 in wheat ash, 207, 237
 range in wheat ash, 212
 ratio to Mg in flour, 206
 salt of phospho-organic acid, 218
 wheat, %, 205, 207, 215, 238
 Calcium sulfate solution, solubility of rye and wheat flour proteins in, 102
 Canadian wheats, riboflavin content, 303
 starch content, 104
 thiamine content, 286, 288, 289, 290, 296
 Carbohydrates, in gliadin, 64
 Carotene, occurrence in flour, 268
 γ -Carotene, 204
 Carotinoids, correlation between content and crops, 272
 correlation in wheat vs. flour, 271, 273
 correlation with wt. per bu., 271
 correlation with wt. per kernel, 273
 Carotinoid pigments, 261 ff
 Casein-like protein (Dumas and Cahours), 15, 16, 22
 Cellulose, in Rumanian wheats, 181
 Chloral hydrate solution, as gliadin solvent, 43
 Chlorine bleaching, effect on chlorine content, 240
 Chlorine, in flours, 212, 215, 240, 242
 in Hungarian flours, 242
 in wheat, 205, 215
 range in wheat ash, 213
 Cholesterin, in germ, 195
 Cholesterol, flour lipoids, 196
 Choline, 193, 199
 in lipoids, 195
 in mill products, 313
 in wheat germ, 132
 Chromatographic fractionation, of carotinoids, 270, 271
 of tocopherols, 315, 316
 Coacervation and gluten, 124
 Cobalt, in bran, 234
 in flour, 215
 in wheat, 215, 234
 Cocarboxylase, 296
 Coefficient of variation of manganese content of wheat, 236
 Congluten (Fluerent), 21
 Converter plants in Se cycle, 246, 247
 Copper, in flours, 212, 215
 in mill products, 210
 in wheat, 207, 215, 232, 233
 in wheat: correlation with soil Cu, 233
 Correlation between, ash and sol. protein, 101
 Greek and AOAC acidity results, 258
 of S vs. P, 244
 of total vs. salt-sol. protein, 97
 Critical peptization temperature of gliadin, 42
 Crude gluten, 106 ff
 composition, 119
 phosphorus in, 121, 221
 proteins of, 61, 63
 selenium in, 252
 toxicity due to Se, 251
 Crude protein, 106 ff
 Cryptoxanthin, in flour, 271
 Crystalline protein, 104, 105
 Cupric hydroxide and protein precipitation, 131, 133
 Curcuma, as indicator in acidity deter., 255
 Cyanogen bromide aniline method of niacin determination, 306, 307
 Cyclic compounds from gliadin, 61
 Cysteine, in wheat germ, 139
 Cystine, in gliadin, 57, 58
 in glutenin, 78, 82, 83, 84, 85
 in wheat, 116
 replacement of S by Se, 250
 Cystine-sulphur, in wheat, 244
 Cytosine, in nucleic acid, 128, 129

 Decalco, adsorption of thiamine, 300
 Dextrin, in wheat and flour, 167
 Dicarboxylic amino acids, in gliadin, 58, 59
 Dihydroergosterol, 204
 Dihydrosterol, 201
 germ lipid, 203
 germ oil, 202
 Dried milk, niacin content, 306
 Durum wheat, see *Triticum durum*

 Edestin, 90
 Einkorn, see *Triticum monococcum*
 Electrodialysis, see electrophoresis
 Electrophoresis of starch, 146, 148, 149, 150, 151
 Electrophoretic studies of gliadin, 70
 Electrostatic charge on gluten micelles, 124
 Elemental analysis of proteins, Liebig, 15
 von Bibra, 16
 Ritthausen, 17, 20
 Osborne, 26, 29, 35
 Embryo, allantoin in, 132
 amids in, 132
 asparagin in, 132
 betain in, 132
 choline in, 132
 glutathione in, 139
 nucleic acids, 123
 oil content, 135
 phosphatidic acid, lecithin and kephalin content, 197
 sugars in, 165, 169
 Embryo oil, 132

- Emmer, see *Triticum dicoccum*
Endosperm, manganese content, 236
 thiamine in, 291
English wheat, riboflavin content, 303
 thiamine content, 280, 286
Ergosterol, 203, 204
 in germ oil, 315
Erythro amylose, 146
Erythrogranulose, 153
d-erythronic acid from starch, 156
Ethanol, as solvent in acidity deter., 256, 257, 259, 260
 as prolamins solvent, 35, 37
Ethylene glycol, solubility of gliadin in, 43
Extinction coefficient and color value, 282
Extraction of flour, effect on thiamine content, 283, 287, 291, 292, 301
 relation to tocopherol content, 316, 317
 thiamine content, 287
Extraction of niacin by acid and alkali solutions, 309, 310
Extraction techniques in acidity deter., 256, 257
- Factor Z, 173
Fats, in flour grades, 183
 in wheat, 184
Fatty acids, of flour, 192
 of starch, 151, 152, 153
 of wheat germ oil, 190, 191, 192
 role in acidity, 259, 260
Feeds, iron in, 230
Fermentation method of thiamine determination, 284, 290
Fertilizers, effect on thiamine content of wheat, 280
Flavones, 274, 275
Flour, biotin content, 314
 bromine content, 242
 choline content, 313
 niacin content, 305, 307, 308, 309, 310
 phosphorus content, 217
 sulfur content, 244
 pyridoxine content, 311
 riboflavin content, 299, 301, 303, 304
 thiamine content, 281
Flour beetles as test animals, 303
Flour extraction, riboflavin content, 301, 302, 303
 thiamine content, 283, 287, 291, 292, 301
Flour grades, amino acids, 136
 niacin content, 312
 pantothenic acid content, 312
 pigments, 278
 pyridoxine content, 311
 reducing matter in, 139
 riboflavin content, 301, 303
 thiamine content, 283, 287, 294
 tocopherol content, 316, 317
Flour quality, relation to Mg content, 237
Flour streams, thiamine content, 294, 296, 298
Fluorometric method for riboflavin determination, 298, 300, 301
Formaldehyde, use as disinfectant in acidity deter., 259
Frosted wheat, non-protein N, 138
Fructose, in bread, 176
 in wheat gliadin, 64
Fructosides, 170, 171
Fungicidal properties of crystalline protein, 105
Furfural from pentosans, 176
- Galactose, from pentosans, 176
 "Gasoline number," 261
Gelatinization temperature of starch, 142
Genetic species of *Triticum*, glutelins from, 80
Genetic species of *Triticum*, lyotropic series of protein solubilities, 96, 97
Genetic species of *Triticum*, proteins of, 48
Gentiobiose linkages in starch, 155
Germ, see embryo
 aluminum content, 234
 calcium content, 238
 Germ—(Continued)
 choline content, 313
 copper content, 238
 fat, composition, 186
 fat content, 185
 inorganic P, 220, 221
 iron in, 231
 lead content, 237
 lecithin in, 195
 lipoids, %, 197, 198
 lipoid P, 226
 manganese content, 235, 236, 237
 minerals of, 210, 211, 212, 214
 niacin content, 306, 307, 308, 309
 nuclein P, 226
 oil constants, 187
 pantothenic acid content, 313
 pellagra-preventive factor, 305
 phosphorus compounds, 224, 226
 phytin P, 226, 228
 pigments of, 269
 pyridoxine in, 310, 311
 raffinose content, 172
 riboflavin content, 299, 300, 303, 304
 silver content, 237
 thiamine content, 281, 282, 289, 291, 292, 295
 tin content, 237
 tocopherol content, 316
 vitamin B content, 281
 vitamin B₂ content, 298
 Germ oil, 182
 tocopherols in, 316
 vitamin E, 314, 315
 German wheat, thiamine content, 284
 Germinated wheat, see malted wheat
 Germination, effect on ascorbic acid content of wheat, 314
Gliadin, 30 ff
 hydrolysis under pressure, 61
 is it a distinct protein, 67
 isoelectric point, 117
 (König and Rintelen), 23
 methods, 34
 (Osborne), 26, 28
 preparation of, 42
 (Ritthausen), 18, 22
 toxicity due to Se, 251
Gliadin-glutenin ratio, 30, 32
Gliadin numbers, 37
Gliadine (Taddei), 12, 22
Glian (O'Brien), 22, 23
Globulin, 90 ff
 definition of, 94
 extracted from wheat species, 97
 from bran, 91
 in wheat, flour, bran, 100
 (Martin), 20, 23
 nitrogen factor, 107
 (O'Brien), 21
 (Osborne), 24, 28
Globulin "A" (Hoffman and Gortner), 93
 "B" (Hoffman and Gortner), 93
Globuline (Weyl), 20, 23
Glucosan, in flour, 173
Glucose, from starch, 154
 in flour, 173
 in phosphatide preps., 196
 in wheat gliadin, 64
Glutamic acid, in gliadin, 59, 61
Glutamine amide from gliadin, 61
Glutathione in wheat germ, 139
Gluten, crude, see crude gluten
Gluten, discovery, 11
 effect of water composition, 121
 electrostatic charge, 124
 fractionation of Se compounds, 250
 isoelectric point, 117
 lipid content, 197
 pre-existence in flour, 124
 proteins in, 31, 61
 selenium content, 248, 249

- Gluten casein (Chittenden and Smith), 21
 (König and Rintelen), 23
 (Ritthausen), 17, 18, 22
 Gluten fibrin (König and Rintelen), 23
 (Martin), 21, 23
 Gluten washing, 118, 231
 Glutelin, 26, 28, 72 ff
 α -Glutelin, 82
 β -Glutelin, 82
 Glutenin, 72 ff
 content of flour, 74, 76
 is it a distinct protein, 67
 isoelectric point, 117
 (Osborne), 26, 28
 Glutenin-gliadin ratio, 30, 32
 Glutin (Dumas and Calours), 14, 15, 22
 (de Saussure), 14, 22
 Glutine (Commaille), 16, 17, 22
 (O'Brien), 22
 (Weyl), 20, 23
 Glycerides, see fats
 Glycerol, solubility of gliadin in, 43
 Glycolipids, 193
 Glycols, solubility of gliadin in, 43
 Greek acidity method, 258
 Guanine, embryo nucleic acid, 128
 Gums, 176 ff
- Halogens, 240 ff
 Harvesting, and amino nitrogen content, 138
 Hausmann nitrogen distribution in gliadin, 60
 Hehner No. of fats, 187
 Hemicellulose, in bran, 177, 180
 in wheat integuments, 179
 of flour, 179
 of starch, 147, 180
 Hexabromide No. of fats, 191
 Histidine, changes during maturation, 136
 in wheat, 116
 Hofmeister lyotropic series, 95
 Hydrocarbons of lipoids, 204
 Hydrocarotin, 200
 Hydrolysis products, of albumin, 103
 of crude gluten, 123
 of gliadin, 55
 of glutenin, 79, 82, 85
 of mesonin, 62
 of proteins, 27
 Hydrolysate of gluten, selenium in, 249
 Hydroxyglutamic acid, in gliadin, 59, 60
 in glutenin, 80
 Hygroscopicity of starch, 165
 Hypoxanthine, in wheat and products, 129
- Immature wheat, proteins from, 92
 Immunochemical studies with proteins, 117, 118
 Indian wheats, niacin content, 307
 thiamine content, 290
 Indicators in acidity deter., 255, 256, 257
 "Indicator Number" in color measurements, 277
 Inesine (fibrin) (Commaille), 16, 17, 22
 Inorganic iron, in bran, 220, 221
 in germ, 220, 221
 Inorganic phosphorus in immature wheat, 219
 in mill products, 227
 in wheat, 220, 221, 224, 226
 Inositol, 218, 222, 223, 225, 226, 228
 International units of thiamine, equivalents in
 other units, 282
 Iodine, in flour, 215
 in mill products, 241
 in wheat, 215, 240, 241
 Iodine No. of fats, 187, 189, 190, 191, 192
 Ionisable iron, in bread, 230
 Ipranol, 202
 Iron, colloidal, as protein precipitant, 133
 Iron, comparison of methods, 229, 231
 in ash, 207
 in bran, 229
 in bread, 230
 in flours, 212, 215
- Iron—(Continued)
 in mill products, 206, 210, 231
 in mill streams, 214
 in Utah wheats, 211
 in wheat, 205, 207, 211, 214, 215, 229 ff
 in wheat ash, 207, 212
 ionisable, 230
 Irrigation, effect on Fe, Cl, and S content of
 wheat, 212
 Isoelectric point, of gliadin, 44, 48, 64, 65, 117
 of gluten, 117
 of glutenins, 88, 117
 of leucosin, 103
 Isoelectric reaction of alcohol-soluble proteins,
 48
 Isopropyl alcohol, as gliadin solvent, 43
- Japanese wheat, iodine content, 241
- Kephalin, 193
 Kephalin, in wheat germ, 197
 Kryptoxanthine, 204
 in germ, 269
- Lactobacillus arabinosus in niacin assays, 309
 Lactobacillus casei in pantothenic assays, 311
 Lactoflavin, see riboflavin
 Lead, in mill products, 237
 in wheat, 237
 Lecithin, 193, 194 ff
 chemical constitution, 196
 cholin-lecithin, 199
 in bran, 195
 in flour, 196
 in mill products, 199, 227
 in mill streams, 217
 in semolinas, 195
 in wheat, 195, 196
 in wheat germ oil, 186
 Lecithin phosphorus, of germ, 214
 of wheat, 226
 Leucine, changes during maturation, 136
 Leucosin (Osborne), 24, 28
 Levosine, 170
 Levulose in bread, 175
 Lignin, in wheat integuments, 179
 Lignoceric acid, wheat germ oil, 190
 Linolenic acid, flour, 192
 wheat germ oil, 190, 191, 193
 Linolic acid, flour, 192
 wheat germ oil, 190, 191, 193
 Lipids, 181 ff
 Lipoid content of wheat, 184, 185
 Lipoid, in germ, 226
 in immature wheat, 219
 phosphorus in, 217, 219, 224, 225, 226
 Lipoprotein of flour, 104, 105
 Lithium, flour, 215
 Lithium chloride as gliadin precipitant, 42, 43
 Litmus, indicator in acidity deter., 256, 257
 Lutein, 204
 in germ, 269, 270
 Lyotropic series of saline solutions, 94, 95
 Lysine in wheat, 116
- Magnesium, in Egyptian wheat, 209
 in flours, 212, 215
 in flour grades, 216
 in mill products, 206, 210
 in mill streams, 214, 237
 in Utah wheats, 211
 in wheat, 205, 215
 in wheat ash, 207, 212, 237
 relation to flour quality, 237
 ratio to Ca in flour, 206
 salt of phospho-organic acid, 218
 Magnesium sulfate solutions, solubility of gli-
 adin in, 46, 47
 Malted wheat, vitamins in, 297
 Maltose, 173, 174

SUBJECT INDEX

- Manganese, in Argentine wheats, 236
 - in bran, 235
 - in flour, 215, 235
 - in flour grades, 216
 - in mill products, 210
 - in wheat, 215
- Manure, effect on thiamine content of wheat, 280
- Maturation of wheat, 219, 220
 - amino acid changes, 133, 137
 - asparagine changes, 136
 - Hausmann No. of proteins, 116
 - manganese content, 235
 - mineral content, 205
 - thiamine changes, 207, 208
- Mesonin, in vulgare and non-vulgare wheats, 63
 - preparation, 62
 - products of hydrolysis, 62
- Methanol, as gliadin solvent, 45
 - as prolamins solvent, 26
- Methionine, in bran globulin and albumin, 104
 - in gliadin, 58
 - replacement of S by Se, 250
- Methyl alcohol as gliadin solvent, 43
- Methyl-ethyl ketone, solubility of gliadin in, 43
- Metol in niacin determination, 307
- Microbiological method, of niacin determination, 307, 308, 309
 - of riboflavin determination, 300, 301, 303, 304
- Microcrystals of starch, 144
- Milk, niacin content, 306
- Mill products, carotinoid content, 272
 - iodine content, 241
 - iron in, %, 231
 - lipid phosphorus in, 220
 - niacin content, 308, 309
 - phosphorus compounds, 227
 - phosphorus in, 220
 - phytosterol in, 220
 - pyridoxine content, 311
 - riboflavin content, 301, 304
 - selenium in, 252
 - S/P ratio, 243, 244
 - thiamine content, 287, 291, 292, 294, 301
 - tocopherol content, 316, 317
- Mill streams, acidity, 258
 - lipid and fat content, 197, 198
 - phosphorus, 217
 - proteins extracted by salt solutions, 98
 - thiamine content, 294, 296
- Mineral analyses, general, 205 ff
- Mineral constituents of starch, 165
- Moisture, hygroscopic, in starch, 165
- Molar binding of gliadin, 66
- Molecular weight, of amyloses, 157
 - of bran and flour fatty acids, 192
 - of crystalline protein, 105
 - of gliadin, 53, 69
 - of glutelins, 89
 - of proteins extracted by salt solutions, 102
 - of starch, 157
- Moving boundary electrophoresis of gliadin, 70
- Mucedin (König and Rintelen), 23
 - (Ritthausen), 19, 22
- Mucin (Berzelius), 14, 22
- Mucine (Commaille), 16, 17, 22
 - (O'Brien), 22
- Myosin (Weyl), 20, 23
- Myxon (O'Brien), 22

- Naphthol yellow as pigment standard, 263
- Niacin, 304 ff
 - effect on *Tribolium*, 310
 - in bran, 234
 - in wheat, 297
 - structural formula, 306
- Nickel, in flour, 215
 - in wheat, 215, 233, 234
- Nicotinic acid, see niacin
- Nitrogen factors for proteins, 106
- Nitrogen fractions in flour, 33
- Nitrogen, in acetone extracts, 189
 - in different kernels of plant, 115
- Nitrogen trichloride, effect on tocopherol in flour, 317
- Non-protein nitrogen, 131 ff
- Nucleic acid, 128 ff
 - phosphorus in, 225
- Nuclein, in germ, 226
 - in mill products, 227
 - in mill streams, 217
 - phosphorus of, germ, 226
- Nucleoproteins, phosphorus in, 225

- Oleic acid
 - flour, 192
 - wheat germ oil, 190, 191, 193

- Palmitic acid, in wheat germ oil, 190, 191
- Palmitoleic acid, in germ oil, 193
- Pantothenic acid, 311 ff
 - changes in malting, 297
 - in wheat, 297
 - structural formula, 312
- Paracholesterol, in lipoids, 185
- Paracresol solutions as prolamins solvents, 26
- Parasitosterol, 200
- Pectic materials, 181
- Pellagra-preventive factor, see niacin
- Pentosans, 176 ff
 - in wheat and flour, %, 167
 - of wheat gum, 178
- Pentoses from hemicelluloses, 181
- Pericarp, manganese content, 236
- Petroleum ether-extract, S in, 245
- Pflanzenleim (von Bibra), 16
 - (Ritthausen), 17
- Phenol, specific rotatory power of gliadin in, 36
- Phenol solutions as prolamins solvents, 26
- Phosphate compounds, role in acidity, 257
- Phosphatides, 393, 394 ff
 - in wheat, %, 196
- Phosphatide-phosphorus, 229
- Phosphatidic acid, 197
- Phospholipids, 194 ff
- Phospho-organic acid, 218
- Phosphorus, correlation with ash, 217
 - in acetone extracts, 189
 - in crude gluten, 121
 - in Egyptian wheats, 209
 - in flours, 212
 - in flour grades, 216
 - in flour phosphatides, 229
 - in French wheats, 226, 227
 - in lecithin, phytin and nuclein, 217
 - in lipoids, 195
 - in mill products, 206, 210
 - in semolinas, 192
 - in starch, 140, 151, 153, 154
 - in Syrian wheats, 227
 - in Utah wheats, 211
 - in wheat, 205, 207, 214
 - range in wheat ash, 213, 237
 - ratio to sulfur in mill products, 243, 244
- Phosphorus compounds, 216 ff
- Phosphotungstic acid precipitation of protein, 132, 133
- Phytalbumose (Martin), 21, 23
- Phytic acid, 218, 219
- Phytic acid salts, 227
- Phytin, in bran, %, 223, 224, 228
 - in flour, 223, 227, 228
 - in germ, 224, 226, 228
 - in immature wheat, 219
 - in mill products, 227
 - in mill streams, 217, 228
 - in wheat, 223, 224, 227, 228
 - methods, 221
 - phosphorus in, 225
 - phosphorus of, germ, 214, 226
 - ratio to P, 223

- Phytosterol, 199
 in germ oil, 202
 in mill products, 220
 in wheat, 196, 202
 Phytosterolin germ, 202
 Plant albumin (Liebig), 14, 22
 (de Saussure), 14, 22
 Plant casein (von Bibra), 16, 22
 Plant fibrin (von Bibra), 16, 22
 (Dumas and Cahours), 15, 16, 22
 (Liebig), 14, 22
 (Scherer), 15, 22
 Plant gelatin (Berzelius), 14, 22
 (von Bibra), 16, 22
 (Liebig), 14, 22
 (Ritthausen), 17, 19, 22
 (de Saussure), 14, 22
 Polarimetric gliadin method, 35, 36
 Polenske No. of fats, 187, 191
 Polish wheat, see *Triticum polonicum*
 Pollard, see shorts
 Potassium, in Egyptian wheats, 209
 in flour, 212, 215
 in flour grades, 216
 in mill products, 205, 206, 210, 211
 in Utah wheats, 211
 in wheat, 205, 215, 239
 range in wheat ash, 212, 237
 Potassium bromide solutions as gliadin peptizers, 46
 Potassium chloride solutions as gliadin peptizers, 46
 Potassium chromate solution as pigment standard, 260, 263
 Potassium iodide solutions as gliadin peptizers, 43, 46
 Potassium, salt of phospho-organic acid, 218
 Potassium thiocyanate as gliadin solvent, 43
 P-P, see niacin
 Precipitin reactions, 117
 Prolamin, 26, 28, 30
 nitrogen factor, 107
 products of hydrolysis, 56, 57
 Prolin, in gliadin, 60
 in glutenin, 80
 n-propyl alcohol as gliadin solvent, 43
 Propylene glycol, solubility of gliadin in, 43
 Protein classification (Osborne), 23
 Protein, alcohol-soluble (Einhof), 12, 22
 correlation between flour ash and sol. protein, 101
 correlation with thiamine content, 285, 288
 crude, 106 ff
 historical, 11, 29
 quality and amino-acid content, 116
 removal of Se from, 251
 replacement of S by Se, 250
 soluble in water, 90 ff
 soluble in salt solutions, 90 ff
 sulfur in, 242, 244
 Protein surveys, König's compilation, 108
 Bailey's analysis, 108
 Zaharia's analyses, 112
 Proteose (O'Brien), 23
 (Osborne), 26
 Purines of wheat nucleic acid, 127, 128
 Pyridoxine, see also vitamin B₆, 310, 311
 structural formula, 311

 Quality of protein and amino-acid content, 116

 Racemization rates, gliadin, 51
 glutelins, 84
 α -glutelin, 84
 β -glutelin, 84
 glutenins, 87
 Raffinose, in wheat germ, 168, 169, 172
 Rat growth, measure of thiamine content, 281, 285
 Rate, effect of Se on growth, 250
 Reagents for precipitating proteins, 133

 Red dog, calcium content, 238
 iodine content, 241
 iron in, 231
 niacin content, 309
 riboflavin content, 301, 304
 thiamine content, 291, 292, 294
 vitamin E, 316
 Reducing matter in flour, 139
 Reducing sugars, in flour, 167, 172, 174
 in wheat, 167, 170
 Refractive index, of fats, 187, 188, 190, 191
 of gliadin solutions, 53
 Refractive index dispersion, gliadin, 53
 glutenins, 86
 Reichert-Meißl No. of fats, 187, 190, 191
 Retrogradation of starch pastes, 145
 Rhamnose, in wheat gliadin, 64
 Riboflavin, changes in malting, 297
 effect on *Tribolium*, 310
 in wheat, 298
 structural formula, 299
 Ribose, in tritico nucleic acid, 129
 Rotation dispersion, gliadin, 51
 glutenin, 86
 Rubixanthin, 204
 Russian wheat, riboflavin content, 303
 Rye flour, identification from sol. protein, 102

 Salt solution-soluble proteins, 90
 Saponification No. of bran and flour fats, 192
 of fats, 186, 187, 189, 190, 191, 192
 of lipids of mill products, 199
 Saskatchewan wheats, selenium content, 253
 Saturated fatty acids (see also palmitic, stearic)
 in bran, 192
 in flour fats, 192
 Scutellum, thiamine content, 292
 Scalins, 177
 Selenium, 245 ff
 converter plants, 246, 247
 effect in soil on Se in wheat crop, 245, 246
 in American soils, 245, 246
 in mill products, 252
 in world wheats, 252 ff
 Semolina, niacin content, 307
 Shorts (middlings)
 aluminum content, 234
 calcium content, 238
 choline content, 313
 copper content, 234
 iodine content, 241
 iron in, 230, 231
 magnesium content, 237
 niacin content, 309
 riboflavin content, 301
 thiamine content, 282, 287, 291, 292, 294
 tocopherol content, 316
 vitamin B content, 281
 Silica, in flour, 215
 range in wheat ash, 213
 Silicon, in wheat, 205, 214
 Silver, in mill products, 237
 in wheat, 237
 Sitesine (Commalle), 16, 17, 22
 Sitosine (Commalle), 16, 17, 22
 Sitosterin, 199
 Sitosterol, in flour, 203
 in germ oil, 202
 in wheat kernel, 202
 Sodium, in flours, 212
 in flour grades, 216
 in mill products, 206, 211
 in wheat, %, 205, 215, 239
 range in wheat ash, 213
 Sodium chloride solution, protein soluble in, 90 ff
 Sodium hydroxide, effect on starch, 142
 Sodium salicylate, dispersion of glutenin in, 77
 effect on starch, 142
 use in fractionating gluten proteins, 68, 69
 Sodium selenate, toxicity to wheat, 248
 Sodium selenite, 249

- Soils, selenium in, 245, 246
 Solvents in acidity deter., 256, 257
 Solvents for extracting carotinoids, 261, 264, 265, 266, 267
 Sorenson formol titration, 131, 133, 135
 Specific rotation equivalent, 52
 Specific rotatory power, amylans, 170
 α -amylose, 154
 β -amylose, 154
 germ sitosterols, 203
 gliadin, 26, 36, 49
 gliadin in various solvents, 36
 α -glutelin, 84
 β -glutelin, 84
 glutenin, 86, 87
 levosine, 170
 nucleic acid, 129
 pentose from bran, 176
 proteins, change on standing in alkali solns., 51
 phytosterol prep., 200, 201
 starch, 154, 162, 164
 unsaponifiable from bran, 201
 wheat and rye gums, 179
 Specific transmissive index of carotinoids, 263, 266
 Spectrographic analysis of flour ash, 215
 Spectrum absorption, glutenin, 86
 Spectrum absorption bands of lipoids, 185
 Spectrum absorption bands, sterols of flour, 204
 Spelt, see *Triticum spelta*
 Sprouted wheat, carbohydrates, 173, 174
 thiamine in, 297
 vitamin C in, 314
 Squalene in germ, 269
 Standard error of thiamine determinations, 287
 Starch, 140 ff
 effect of NaOH on, 142
 effect of sodium salicylate on, 142
 fatty acids in, 151, 152, 153
 gelatinization temperatures, 142
 in flour, %, 161
 in mill offals, 164
 in wheat, %, 164, 165, 167
 in wheat products, %, 164, 165
 mineral constituents, 165
 microcrystals of, 144
 molecular weight, 157
 phosphorus in, 142, 221, 225
 retrogradation of pastes, 145
 thermal fractionation, 147
 x-ray spectra, 144, 145
 Starch granules, size, 140
 Shape, 141
 structure, 141, 143
 Stearic acid, wheat germ oil, 190, 191
 Stems, potassium content, 239
 sodium content, 239
 Sterols, 199 ff
 germ oil, 314, 315
 wheat fats, %, 203
 Stone ground flour, thiamine content, 295
 Storage, change in acidity, 258, 260
 change in carotinoid pigment content, 264
 Straw, gum from, 177
 Stutzer's method, 131, 133
 Sucrose, in flour, 168
 in various portions of kernel, 168
 in wheat, 167, 168, 170
 Sugars, 167 ff
 in wheat gliadin, 64
 Sulfhydryl groups in lipoids, 194
 Sulfur, 242 ff
 effect on Se absorption by wheat, 247, 248
 in flours, 212, 215
 in glutenins, 84
 in lipoids, 194
 in mill products, 243
 in Utah wheats, 211
 in wheat, 205, 207, 215
 Sulfur—(Continued)
 ratio to P in mill products, 243
 ratio to protein, 242
 Supersorb, adsorption of riboflavin, 300
 Surface tension of fats, 190
 Synanthrose, 169
 Tannic acid as protein precipitant, 133
 Temperature of starch gelatinization, 142
 Temperature, relation to protein solubility in alcohol, 36, 38, 39, 42
 Tempering, changes in amino compounds, 137
 Thermal fractionation, of proteins, 47, 70
 of starch, 147
 Thiamine, changes in malting, 297
 changes in maturation of wheat, 297, 298
 conversion factors for various units, 282
 correlation with protein and ash content, 285, 288
 in Australian flours, 287
 in wheat, 286
 structural formula, 281
 Thiamine determinations, accuracy of, 287, 289
 Thiochrome determination of thiamine content, 285, 286
 Thiocyanogen No. of fats, 191
 Tin, in flour, 215
 in mill products, 237
 in wheat, 237
 Titanium, flour, 215
 wheat, 215
 Tocopherol, see vitamin E
 chromatographic fractionation, 315, 316
 structural formula, 315
 Tribolium confusum, as test animals, 303
 effect of riboflavin in diet, 310
 effect of vitamin B₆ in diet, 310
 niacin essential for growth, 310
 Triectin, 275
 Trichloroacetic acid, as protein precipitant, 133
 Trichloroethylene as lipid solvent, 204
 Tricin, 275
 Trigonellin, 196
 Tritico nucleic acid, 128, 225
 Triticum compactum, lyotropic series of protein solubilities, 97
 Triticum dicoccoides, sugars, 170
 Triticum dicoccum, glutenin, 80
 lyotropic series of protein solubilities, 97
 prolamin, 58
 sugars in gliadin, 64
 Triticum durum, acetone extract, 189
 carotinoid content, 272
 composition of gluten, 121
 fat constants, 190
 glutenin from, 80
 gum from, 178
 lyotropic series of protein solubilities, 97
 phytosterol of, 203
 prolamin, 58
 proteins of, 48
 starch characteristics, 143
 starch content, 164
 sterols, 203
 thiamine content, 286, 291
 tocopherol in, 316
 Triticum monococcum, glutenin from, 80
 lyotropic series of protein solubilities, 97
 prolamin, 58
 proteins of, 48
 sugars in gliadin, 64
 Triticum polonicum, lyotropic series of protein solubilities, 97
 proteins of, 48
 sugars in, 170
 Triticum spelta, glutenin from, 80
 prolamin, 58
 sugars in gliadin, 64
 Triticum turgidum, lyotropic series of protein solubilities, 97

- Tryptophane, in gliadin, 57, 58
 in glutenin, 78, 82, 83
 in protein fractions, 48
 in water- and salt-sol. proteins, 104
 in wheat, 116
 Tryptophane-like compound, Se in, 250
 Turmeric indicator in acidity deter., 256, 257
 Tyrosine, in gliadin, 57, 58
 in α -glutelin, 83
 in protein fractions, 48
 in water- and salt-sol. proteins, 104
 in wheat, 116

 Ultra centrifugal methods, gliadin preparations, 69, 70
 Unsaponifiable matter, in acetone extracts, 189
 in bran oil, 192, 201
 in crude and refined oil, 201
 in flour fats, 191, 192
 in germ lipid, 191, 203
 in lipoids of mill products, 199
 Uracil, in nucleic acid, 128
 Urea solutions, as gliadin solvent, 43
 as gluten solvent, 80
 as glutenin solvent, 77
 dissociation of gliadin in, 53
 effect on starch, 142
 in protein fractionation, 46

 Van Slyke fractionation, albumins, 103
 gliadin, 56, 57
 globulins, 103
 α -glutelin, 83
 β -glutelin, 83
 glutenin, 79, 85
 Viscosity of gliadin solutions, 48
 of starch suspensions, 142
 Vitamins, 280 ff
 Vitamin A, 280
 Vitamin B complex, 280, 281, 305
 distribution in wheat, 282
 Vitamin B₁, see thiamine
 Vitamin B₂, see riboflavin
 Vitamin B₆, see pyridoxine
 effect on tribolium, 810
 Vitamin C, see ascorbic acid
 Vitamin E, 314, 315
 Vitamin E, in germ unsaponifiable, 203
 Vitamin G, see riboflavin
 Vitamin H, see biotin

 Water-soluble proteins, 90
 (Ritthausen), 20, 22
 Wheat, ascorbic acid content, 314
 biotin content, 313, 314
 bromine content, 242

 Wheat—(Continued)
 calcium content, 238
 carotinoid pigment content, 265, 271
 carotinoids extracted by butanol, 273
 chlorine content, 240
 choline content, 313
 copper in, 233
 iron content, 231, 232
 lead content, 237
 manganese content, 235
 manganese content: coefficient of variation, 236
 minerals of, 205, 206, 207, 208, 209, 210, 211
 niacin content, 305, 306, 307, 308, 309, 310
 nickel content, 233, 234
 phosphorus content, 217
 potassium content, 239
 pyridoxine content, 310, 311
 riboflavin content, 299, 300, 301, 302, 303, 304
 selenium content, 252 ff
 sodium content, 239
 sulfur content, 242, 243
 thiamine content, 281, 283, 284, 285, 286, 287, 288,
 289, 290, 291, 296, 297
 tin content, 237
 tocopherol content, 317
 zinc content, 234
 Wheat classes, niacin content, 309
 pantothenic acid content, 312
 pyridoxine content, 310, 311
 riboflavin content, 300, 302
 thiamine content, 286, 291
 Wheat plant, thiamine in parts, 297
 tolerance to Se, 247
 Wheat varieties, niacin content, 308
 pyridoxine content, 310
 riboflavin content, 300, 301, 302
 thiamine content, 285, 286, 289
 Whole wheat flour, niacin content, 310
 thiamine content, 284, 290

 Xanthine in wheat and products, 129
 Xanthophylls, occurrence in flour, 286, 269, 270,
 271
 X-ray spectra of starch, 144, 145, 154, 155
 Xylose from pentosans, 176, 177

 Yeast, niacin content, 306

 Z-factor, 173
 Zimome (Taddei), 12, 22
 Zinc, in bran, 234
 in flour, 215
 in mill products, 210
 in wheat, 215, 234
 Zymom (O'Brien), 22, 23

American Chemical Society
MONOGRAPH SERIES
PUBLISHED

No.

1. **The Chemistry of Enzyme Action (Revised Edition).** By K. George Falk.
2. **The Chemical Effects of Alpha Particles and Electrons (Revised Edition).** By Samuel C. Lind.
3. **Organic Compounds of Mercury.** By Frank C. Whitmore. (Out of Print)
4. **Industrial Hydrogen.** By Hugh S. Taylor. (Out of Print)
5. **Zirconium and Its Compounds.** By Francis P. Venable.
6. **The Vitamins (Revised Edition).** By H. C. Sherman and S. L. Smith.
7. **The Properties of Electrically Conducting Systems.** By Charles A. Kraus.
8. **The Origin of Spectra.** By Paul D. Foote and F. L. Mohler. (Out of Print)
9. **Carotinoids and Related Pigments.** By Leroy S. Palmer.
10. **The Analysis of Rubber.** By John B. Tuttle. (Out of Print)
11. **Glue and Gelatin.** By Jerome Alexander. (Out of Print)
12. **The Chemistry of Leather Manufacture (Revised Edition).** By John A. Wilson. Vol. I and Vol. II.
13. **Wood Distillation.** By L. F. Hawley. (Out of Print)
14. **Valence and the Structure of Atoms and Molecules.** By Gilbert N. Lewis. (Out of Print)
15. **Organic Arsenical Compounds.** By George W. Raiziss and Jos. L. Gavron.
16. **Colloid Chemistry (Revised Edition).** By The Svedberg.
17. **Solubility (Revised Edition).** By Joel H. Hildebrand.
18. **Coal Carbonization.** By Horace C. Porter. (Revision in preparation)
19. **The Structure of Crystals (Second Edition) and Supplement to Second Edition.** By Ralph W. G. Wyckoff.
20. **The Recovery of Gasoline from Natural Gas.** By George A. Burrell. (Out of Print)
21. **The Chemical Aspects of Immunity (Revised Edition).** By H. Gideon Wells.
22. **Molybdenum, Cerium and Related Alloy Steels.** By H. W. Gillett and E. L. Mack.
23. **The Animal as a Converter of Matter and Energy.** By H. P. Armsby and C. Robert Moulton.
24. **Organic Derivatives of Antimony.** By Walter G. Christiansen.
25. **Shale Oil.** By Ralph H. McKee.
26. **The Chemistry of Wheat Flour.** By C. H. Bailey.
27. **Surface Equilibria of Biological and Organic Colloids.** By P. Lecomte du Noüy.
28. **The Chemistry of Wood.** By L. F. Hawley and Louis E. Wise. (Out of print)
29. **Photosynthesis.** By H. A. Spoehr. (Out of Print)

American Chemical Society Monograph Series

PUBLISHED

No.

30. **Casein and Its Industrial Application (Revised Edition).** By Ewin Sutermeister and F. L. Browne.
31. **Equilibria in Saturated Salt Solutions.** By Walter C. Blasdale.
32. **Statistical Mechanics as Applied to Physics and Chemistry.** By Richard C. Tolman. (Out of Print)
33. **Titanium.** By William M. Thornton, Jr.
34. **Phosphoric Acid, Phosphates and Phosphatic Fertilizers.** By W. H. Waggaman.
35. **Noxious Gases.** By Yandell Henderson and H. W. Haggard. (Revised Edition)
36. **Hydrochloric Acid and Sodium Sulfate.** By N. A. Laury.
37. **The Properties of Silica.** By Robert B. Sosman.
38. **The Chemistry of Water and Sewage Treatment.** By Arthur M. Buswell. (Revision in preparation)
39. **The Mechanism of Homogeneous Organic Reactions.** By Francis O. Rice.
40. **Protective Metallic Coatings.** By Henry S. Rawdon. Replaced by **Protective Coatings for Metals.**
41. **Fundamentals of Dairy Science (Revised Edition).** By Associates of Rogers.
42. **The Modern Calorimeter.** By Walter P. White.
43. **Photochemical Processes.** By George Kistiakowsky.
44. **Glycerol and the Glycols.** By James W. Lawrie.
45. **Molecular Rearrangements.** By C. W. Porter
46. **Soluble Silicates in Industry.** By James G. Vail.
47. **Thyroxine.** By E. C. Kendall.
48. **The Biochemistry of the Amino Acids.** By H. H. Mitchell and T. S. Hamilton. (Revision in preparation)
49. **The Industrial Development of Searles Lake Brines.** By John E. Teeple.
50. **The Pyrolysis of Carbon Compounds.** By Charles D. Hurd.
51. **Tin.** By Charles L. Mantell.
52. **Diatomaceous Earth.** By Robert Calvert.
53. **Bearing Metals and Bearings.** By William M. Corse.
54. **Development of Physiological Chemistry in the United States.** By Russell H. Chittenden.
55. **Dielectric Constants and Molecular Structure.** By Charles P. Smyth. (Out of Print)
56. **Nucleic Acids.** By P. A. Levene and L. W. Bass.
57. **The Kinetics of Homogeneous Gas Reactions.** By Louis S. Kassel.
58. **Vegetable Fats and Oils (Revised edition).** By George S. Jamieson
59. **Fixed Nitrogen.** By Harry A. Curtis.
60. **The Free Energies of Some Organic Compounds.** By G. S. Parks and H. M. Huffman. (Out of Print)
61. **The Catalytic Oxidation of Organic Compounds in the Vapor Phase.** B. L. F. Marek and Dorothy A. Hahn.

American Chemical Society Monograph Series

PUBLISHED

No.

62. **Physiological Effects of Radiant Energy.** By H. Laurens.
63. **Chemical Refining of Petroleum.** By Kalichevsky and B. A. Stagner. (Second Edition)
64. **Therapeutic Agents of the Quinoline Group.** By W. F. Von Oettingen.
65. **Manufacture of Soda.** By T. P. Hou. (Revised Edition)
66. **Electrokinetic Phenomena and Their Application to Biology and Medicine.** By H. A. Abramson.
67. **Arsenical and Argentiferous Copper.** By J. L. Gregg.
68. **Nitrogen System of Compounds.** By E. C. Franklin.
69. **Sulfuric Acid Manufacture.** By Andrew M. Fairlie.
70. **The Chemistry of Natural Products Related to Phenanthrene (Second Edition with Appendix).** By L. F. Fieser.
71. **The Corrosion Resistance of Metals and Alloys.** By Robert J. McKay and Robert Worthington.
72. **Carbon Dioxide.** By Elton L. Quinn and Charles L. Jones.
73. **The Reactions of Pure Hydrocarbons.** By Gustav Egloff.
74. **Chemistry and Technology of Rubber.** By C. C. Davis and J. T. Blake.
75. **Polymerization.** By R. E. Burk, A. J. Weith, H. E. Thompson and I. Williams.
76. **Modern Methods of Refining Lubricating Oils.** By V. A. Kalichevsky.
77. **Properties of Glass.** By George W. Morey.
78. **Physical Constants of Hydrocarbons.** By Gustav Egloff. Vols. I and II.
79. **Protective Coatings for Metals.** By R. M. Burns and A. E. Schuh.
80. **Raman Effect and its Chemical Applications.** By James H. Hibben.
81. **Properties of Water.** By Dr. N. E. Dorsey.
82. **Mineral Metabolism.** By A. T. Shohl.
83. **Phenomena at the Temperature of Liquid Helium.** By E. F. Burton, H. Grayson Smith and J. O. Wilhelm.
84. **The Ring Index.** By A. M. Patterson and L. T. Capell.
85. **Photodynamic Action and Diseases Caused by Light.** By Harold Francis Blum.
86. **Photochemistry of Gases.** W. A. Noyes and P. L. Leighton.
87. **Anhydrous Aluminum Chloride in Organic Chemistry.** By Charles A. Thomas.
88. **Isomerization of Pure Hydrocarbons.** By Gustav Egloff, V. I. Komarevsky, and G. Hulla.
89. **Chemistry of Natural Coloring Matters.** By F. Mayer.
90. **Proteins, Amino Acids and Peptides.** By E. J. Cohn and J. T. Edsall.
91. **Potash in North America.** By J. W. Turrentine.
92. **The Chemistry of Aliphatic Orthoesters.** By Howard W. Post.
93. **The Biochemistry of the Fats and Related Substances.** By W. R. Bloor.
94. **Tungsten.** K. C. Li and C. Y. Wang.
95. **Physical Chemistry of Electrolytic Solutions.** By H. E. Harned and B. B. Owen.

American Chemical Society Monograph Series

IN PREPARATION

Piezo-Chemistry. By L. H. Adams.

The Chemistry of Coordinate Compounds. By J. C. Bailar.

Water Softening. By A. S. Behrman.

Proteins in Metabolism and Nutrition. By George E. Cowgill.

The Refining of Motor Fuels. By G. Egloff.

Organometallic Compounds. By Henry Gilman.

Animal and Vegetable Waxes. By L. W. Greene.

Surface Energy and Colloidal Systems. By W. D. Harkins and T. F. Young.

Raw Materials of Lacquer Manufacture. By J. S. Long.

The Chemistry of Acetylene. By J. A. Nieuwland and R. R. Vogt.

Furfural and other Furan Compounds. By F. N. Peters, Jr., and H. J. Brownlee.

Aliphatic Sulfur Compounds. By E. Emmet Reid.

Electrical Precipitation of Suspended Particles from Gases. By W. A. Schmidt and Evald Anderson.

Dipole Moments. By C. P. Smyth.

Formaldehyde. By J. Frederic Walker.

Precise Electric Thermometry. By W. P. White and E. F. Mueller.

Colloidal Carbon. By W. B. Wiegand.

The Chemistry of Leather Manufacture. (Supplement.) By E. R. Theis and G. D. McLaughlin.

Measurement of Particle Size and Its Application. By L. T. Work.

Physical Constants of Hydrocarbons. Vol. III. By G. Egloff.

Wood Chemistry. Edited by Louis E. Wise.

Alkaline-earth and Heavy-metal Soaps. By S. B. Elliott.

DATE OF ISSUE

This book must be returned
within 3, 7, 14 days of its issue. A
fine of ONE ANNA per day will
be charged if the book is overdue.

--	--

